

MAMMALIAN CYTOKINES; RELATED REAGENTS AND METHODS

This filing is a conversion of U.S. Provisional Patent
5 Applications USSN 60/131, 298, filed April 27, 1999, and
USSN 60/101,318, filed September 21, 1998, each of which is
incorporated herein by reference, to a U.S. Utility Patent
Application.

10 FIELD OF THE INVENTION

The present invention pertains to compositions related
to proteins which function in controlling biology and
physiology of mammalian cells, e.g., cells of a mammalian
immune system. In particular, it provides purified genes,
15 proteins, antibodies, and related reagents useful, e.g., to
regulate activation, development, differentiation, and
function of various cell types, including hematopoietic
cells.

20 BACKGROUND OF THE INVENTION

Recombinant DNA technology refers generally to the
technique of integrating genetic information from a donor
source into vectors for subsequent processing, such as
through introduction into a host, whereby the transferred
25 genetic information is copied and/or expressed in the new
environment. Commonly, the genetic information exists in
the form of complementary DNA (cDNA) derived from messenger
RNA (mRNA) coding for a desired protein product. The
carrier is frequently a plasmid having the capacity to
30 incorporate cDNA for later replication in a host and, in
some cases, actually to control expression of the cDNA and
thereby direct synthesis of the encoded product in the host.

For some time, it has been known that the mammalian
immune response is based on a series of complex cellular
35 interactions, called the "immune network". Recent research
has provided new insights into the inner workings of this

network. While it remains clear that much of the response does, in fact, revolve around the network-like interactions of lymphocytes, macrophages, granulocytes, and other cells, immunologists now generally hold the opinion that soluble
5 proteins, known as lymphokines, cytokines, or monokines, play a critical role in controlling these cellular interactions. Thus, there is considerable interest in the isolation, characterization, and mechanisms of action of cell modulatory factors, an understanding of which will lead
10 to significant advancements in the diagnosis and therapy of numerous medical abnormalities, e.g., immune system disorders. Some of these factors are hematopoietic growth and/or differentiation factors, e.g., stem cell factor (SCF) and IL-7. See, e.g., Mire-Sluis and Thorpe (1998) Cytokines
15 Academic Press, San Diego; Thomson (ed. 1998) The Cytokine Handbook (3d ed.) Academic Press, San Diego; Metcalf and Nicola (1995) The Hematopoietic Colony Stimulating Factors Cambridge University Press; and Aggarwal and Gutterman (1991) Human Cytokines Blackwell.

20 Lymphokines apparently mediate cellular activities in a variety of ways. They have been shown to support the proliferation, growth, and differentiation of pluripotential hematopoietic stem cells into vast numbers of progenitors comprising diverse cellular lineages making up a complex
25 immune system. Proper and balanced interactions between the cellular components are necessary for a healthy immune response. The different cellular lineages often respond in a different manner when lymphokines are administered in conjunction with other agents.

30 Cell lineages especially important to the immune response include two classes of lymphocytes: B-cells, which can produce and secrete immunoglobulins (proteins with the capability of recognizing and binding to foreign matter to effect its removal), and T-cells of various subsets that
35 secrete lymphokines and induce or suppress the B-cells and various other cells (including other T-cells) making up the

immune network. These lymphocytes interact with many other cell types.

Another important cell lineage is the mast cell (which has not been positively identified in all mammalian species), which is a granule-containing connective tissue cell located proximal to capillaries throughout the body. These cells are found in especially high concentrations in the lungs, skin, and gastrointestinal and genitourinary tracts. Mast cells play a central role in allergy-related disorders, particularly anaphylaxis as follows: when selected antigens crosslink one class of immunoglobulins bound to receptors on the mast cell surface, the mast cell degranulates and releases mediators, e.g., histamine, serotonin, heparin, and prostaglandins, which cause allergic reactions, e.g., anaphylaxis.

Research to better understand and treat various immune disorders has been hampered by the general inability to maintain cells of the immune system in vitro. Immunologists have discovered that culturing these cells can be accomplished through the use of T-cell and other cell supernatants, which contain various growth factors, including many of the lymphokines.

From the foregoing, it is evident that the discovery and development of new lymphokines, e.g., related to IL-7, could contribute to new therapies for a wide range of degenerative or abnormal conditions which directly or indirectly involve the immune system and/or hematopoietic cells. In particular, the discovery and development of lymphokines which enhance or potentiate the beneficial activities of known lymphokines would be highly advantageous. The present invention provides new interleukin compositions and related compounds, and methods for their use.

SUMMARY OF THE INVENTION

The present invention is directed to mammalian, e.g., rodent, canine, feline, primate, interleukin-B50 (IL-B50) and its biological activities. It includes nucleic acids coding for polypeptides themselves and methods for their production and use. The nucleic acids of the invention are characterized, in part, by their homology to cloned complementary DNA (cDNA) sequences enclosed herein, and/or by functional assays for growth factor- or cytokine-like activities, e.g., IL-7 (see Maeurer, et al. (1998) in Thomson (ed.) The Cytokine Handbook 3d ed., Academic Press, San Diego; Namien and Mire-Sluis (1998) in Mire-Sluis and Thorpe (eds.) Cytokines Academic Press, San Diego; and Edington and Lotze (1994) in Thomson (ed.) The Cytokine Handbook 2d ed., Academic Press, San Diego), applied to the polypeptides, which are typically encoded by these nucleic acids. Methods for modulating or intervening in the control of a growth factor dependent physiology or an immune response are provided.

The present invention is based, in part, upon the discovery of a new cytokine sequence exhibiting significant sequence and structural similarity to IL-7. In particular, it provides primate, e.g., human, sequences. Functional equivalents exhibiting significant sequence homology will be available from other mammalian, e.g., cow, horse, and rat, mouse, and non-mammalian species.

In various protein embodiments, the invention provides: a substantially pure or recombinant IL-B50 polypeptide exhibiting identity over a length of at least about 12 amino acids to SEQ ID NO: 2 or 4; a natural sequence IL-B50 of SEQ ID NO: 2 or 4; and a fusion protein comprising IL-B50 sequence. In certain embodiments, the segment of identity is at least about 14, 17, or 19 amino acids. In other embodiments, the IL-B50 comprises a mature sequence comprising the sequence from Table 1; or exhibits a post-translational modification pattern distinct from

natural IL-B50; or the polypeptide: is from a warm blooded animal selected from a mammal, including a primate; comprises at least one polypeptide segment of SEQ ID NO: 2 or 4; exhibits a plurality of fragments; is a natural allelic variant of IL-B50; has a length at least about 30 amino acids; exhibits at least two non-overlapping epitopes which are specific for a primate IL-B50; exhibits sequence identity over a length of at least about 20 amino acids to primate IL-B50; is glycosylated; has a molecular weight of at least 10 kD with natural glycosylation; is a synthetic polypeptide; is attached to a solid substrate; is conjugated to another chemical moiety; is a 5-fold or less substitution from natural sequence; or is a deletion or insertion variant from a natural sequence. Preferred embodiments include a composition comprising: a sterile IL-B50 polypeptide; or the IL-B50 polypeptide and a carrier, wherein the carrier is: an aqueous compound, including water, saline, and/or buffer; and/or formulated for oral, rectal, nasal, topical, or parenteral administration. In fusion protein embodiments, the protein can have: mature polypeptide sequence from Table 1; a detection or purification tag, including a FLAG, His6, or Ig sequence; and/or sequence of another cytokine or chemokine, including an IL-7.

Kit embodiments include those with an IL-B50 polypeptide, and: a compartment comprising the polypeptide; and/or instructions for use or disposal of reagents in the kit.

In binding compound embodiments, the compound may have an antigen binding site from an antibody, which specifically binds to a natural IL-B50 polypeptide, wherein: the IL-B50 is a primate protein; the binding compound is an Fv, Fab, or Fab2 fragment; the binding compound is conjugated to another chemical moiety; or the antibody: is raised against a peptide sequence of a mature polypeptide portion from Table 1; is raised against a mature IL-B50; is raised to a purified primate IL-B50; is immunoselected; is a polyclonal

antibody; binds to a denatured IL-B50; exhibits a K_d of at least 30 μM ; is attached to a solid substrate, including a bead or plastic membrane; is in a sterile composition; or is detectably labeled, including a radioactive or fluorescent

5 label. Kits containing binding compounds include those with: a compartment comprising the binding compound; and/or instructions for use or disposal of reagents in the kit.

Often the kit is capable of making a qualitative or quantitative analysis. Preferred compositions will

10 comprise: a sterile binding compound; or the binding compound and a carrier, wherein the carrier is: an aqueous compound, including water, saline, and/or buffer; and/or formulated for oral, rectal, nasal, topical, or parenteral administration.

15 Nucleic acid embodiments include an isolated or recombinant nucleic acid encoding an IL-B50 polypeptide or fusion protein, wherein: the IL-B50 is from a primate; and/or the nucleic acid: encodes an antigenic peptide sequence of Table 1; encodes a plurality of antigenic
20 peptide sequences of Table 1; exhibits identity to a natural cDNA encoding the segment; is an expression vector; further comprises an origin of replication; is from a natural source; comprises a detectable label; comprises synthetic nucleotide sequence; is less than 6 kb, preferably less than
25 3 kb; is from a primate, including a human; comprises a natural full length coding sequence; is a hybridization probe for a gene encoding the IL-B50; or is a PCR primer, PCR product, or mutagenesis primer. The invention also provides a cell, tissue, or organ comprising such a
30 recombinant nucleic acid, and preferably the cell will be: a prokaryotic cell; a eukaryotic cell; a bacterial cell; a yeast cell; an insect cell; a mammalian cell; a mouse cell; a primate cell; or a human cell.

Kit embodiments include those with such nucleic acids,
35 and: a compartment comprising the nucleic acid; a compartment further comprising the IL-B50 protein or

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polypeptide; and/or instructions for use or disposal of reagents in the kit. Typically, the kit is capable of making a qualitative or quantitative analysis.

In certain embodiments, the nucleic acid: hybridizes
5 under wash conditions of 30° C and less than 2M salt, or of
45° C and/or 500 mM salt, or 55° C and/or 150 mM salt, to
SEQ ID NO: 1 or 3; or exhibits identity over a stretch of at
least about 30, 55, or 75 nucleotides, to a primate IL-B50.

The invention embraces a method of modulating
10 physiology or development of a cell or tissue culture cells
comprising contacting the cell with an agonist or antagonist
of a primate IL-B50. The method may be where: the
contacting is in combination with an agonist or antagonist
of IL-7; or the contacting is with an antagonist, including
15 a binding composition comprising an antibody binding site
which specifically binds an IL-B50.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

All references cited herein are incorporated herein by reference to the same extent as if each individual
5 publication or patent application was specifically and individually indicated to be incorporated by reference.

OUTLINE

- 10 I. General
II. Purified IL-B50
A. physical properties
B. biological properties
15 III. Physical Variants
A. sequence variants, fragments
B. post-translational variants
1. glycosylation
2. others
IV. Functional Variants
20 A. analogs, fragments
1. agonists
2. antagonists
B. mimetics
1. protein
25 C. species variants
V. Antibodies
A. polyclonal
B. monoclonal
30 C. fragments, binding compositions
VI. Nucleic Acids
A. natural isolates; methods
B. synthetic genes
C. methods to isolate
35 VII. Making IL-B50, mimetics
A. recombinant methods
B. synthetic methods
C. natural purification
VIII. Uses
40 A. diagnostic
B. therapeutic
IX. Kits
A. nucleic acid reagents
B. protein reagents
45 C. antibody reagents
X. Isolating receptors for IL-B50

I. General

The present invention provides amino acid sequences and DNA sequences encoding various mammalian proteins which are cytokines, e.g., which are secreted molecules which can mediate a signal between immune or other cells. See, e.g., Paul (1997) Fundamental Immunology (3d ed.) Raven Press, N.Y. The full length cytokines, and fragments, or antagonists will be useful in physiological modulation of cells expressing a receptor. It is likely that IL-B50 has either stimulatory or inhibitory effects on hematopoietic cells, including, e.g., lymphoid cells, such as T-cells, B-cells, natural killer (NK) cells, macrophages, dendritic cells, hematopoietic progenitors, etc. The proteins will also be useful as antigens, e.g., immunogens, for raising antibodies to various epitopes on the protein, both linear and conformational epitopes.

A cDNA encoding IL-B50 was identified from a human cDNA sequence. See, e.g., Accession number AA889581. The molecule was designated huIL-B50.

The human gene will encode a small soluble cytokine-like protein, of about 175 amino acids. The signal sequence probably is about 33 residues, and would run from the met to about thr. See Table 1 and SEQ ID. NO: 1 and 2; supplementary sequence provides SEQ ID NO: 3 and 4. IL-B50 exhibits structural motifs characteristic of a member of the short chain cytokines. Compare, e.g., IL-B50 and IL-7, sequences available from GenBank. See also Table 2.

	AGTGTGAAAC	TGGGGTGGAA	TGGGGTGTCC	ACGTATGTTT	CCTTTTGCCT	TACTATATGT	60
5	TCTGTCAAGT	TCTTTTCAAG	AAATCTTCAT	CTTACAACCT	GTAGGGCTGG	TGTTAACTTA	120
	CGACTTCACT	AACTGTGACT	TTGAGAAGAT	TAAAGCAGCC	TATCTCAGTA	CTATTTCTAA	180
	AGACCTGATT	ACATATATGA	GTGGGACCAA	AAGTACCGAG	TTCAACAACA	CCGTCTCTTG	240
	TAGCAATCGG	CCACATTGCC	TTACTGAAAT	CCAGAGCCTA	ACCTTCAATC	CCAACCGCCG	300
10	NGTGCGGTCG	CTCGCCAAAAG	AAATGTTCGC	CATGAAAAC	AAGGCTGCCT	TAGCTATCTG	360
	GTGCCCAGGC	TATTTCGGAAA	CTCAGATAAA	TGCTACTCAG	GCAATGAAGA	AGAGGAGAAA	420
	AAGGAAAGTC	ACAACCAATA	AATGTCTGGA	ACAAGTGTC	CAATTTAAA		468
	agtgtgaaac	tgggggtgga	atg ggg tgt	cca cgt atg	ttc cct ttt	gcc tta	52
15			Met Gly Cys	Pro Arg Met	Phe Pro Phe	Ala Leu	
			-30		-25		
	cta tat gtt	ctg tca gtt	tct ttc agg	aaa atc ttc	atc tta caa	ctt	100
	Leu Tyr Val	Leu Ser Val	Ser Phe Arg	Lys Ile Phe	Ile Leu Gln	Leu	
	-20		-15		-10		
20	gta ggg ctg	gtg tta act	tac gac ttc	act aac tgt	gac ttt gag	aag	148
	Val Gly Leu	Val Leu Thr	Tyr Asp Phe	Thr Asn Cys	Asp Phe Glu	Lys	
	-5	-1	1	5	10		
25	att aaa gca	gcc tat ctc	agt act att	tct aaa gac	ctg att aca	tat	196
	Ile Lys Ala	Ala Tyr Leu	Ser Thr Ile	Ser Lys Asp	Leu Ile Thr	Tyr	
		15		20	25		
30	atg agt ggg	acc aaa agt	acc gag ttc	aac aac acc	gtc tct tgt	agc	244
	Met Ser Gly	Thr Lys Ser	Thr Glu Phe	Asn Asn Thr	Val Ser Cys	Ser	
		30	35		40		
35	aat cgg cca	cat tgc ctt	act gaa atc	cag agc cta	acc ttc aat	ccc	292
	Asn Arg Pro	His Cys Leu	Thr Glu Ile	Gln Ser Leu	Thr Phe Asn	Pro	
	45		50		55		
40	aac cgc cgn	gtg cgg tgc	ctc gcc aaa	gaa atg ttc	gcc atg aaa	act	340
	Asn Arg Xaa	Val Arg Ser	Leu Ala Lys	Glu Met Phe	Ala Met Lys	Thr	
	60		65		70		
45	aag gct gcc	tta gct atc	tgg tgc cca	ggc tat tgc	gaa act cag	ata	388
	Lys Ala Ala	Leu Ala Ile	Trp Cys Pro	Gly Tyr Ser	Glu Thr Gln	Ile	
	75	80		85		90	
50	aat gct act	cag gca atg	aag aag agg	aga aaa agg	aaa gtc aca	acc	436
	Asn Ala Thr	Gln Ala Met	Lys Lys Arg	Arg Lys Arg	Lys Val Thr	Thr	
		95		100		105	
55	aat aaa tgt	ctg gaa caa	gtg tca caa	tta aa			468
	Asn Lys Cys	Leu Glu Gln	Val Ser Gln	Leu			
		110		115			
	<u>MGCPRMFPPALLYVLSVSFRKIFILOLVGLVLT</u>						
	YDFTNCDFEKIKAAYLSTISKDLITYMSGTKSTEFNNTVSCSNRPHCLTEIQSLTFNPNRXVRS						LAKEM
	FAMKTKAALAIWCPGYSETQINATOAMKKRRKRKVT						TNNKCLEOV
							SOL

Supplemental primate, e.g., human, sequence (SEQ ID NO: 3 and 4);
predicted signal cleavage position indicated:

5	ATG TTC CCT TTT GCC TTA CTA TAT GTT CTG TCA GTT TCT TTC AGG AAA	48
	Met Phe Pro Phe Ala Leu Leu Tyr Val Leu Ser Val Ser Phe Arg Lys	
	-28 -25 -20 -15	
10	ATC TTC ATC TTA CAA CTT GTA GGG CTG GTG TTA ACT TAC GAC TTC ACT	96
	Ile Phe Ile Leu Gln Leu Val Gly Leu Val Leu Thr Tyr Asp Phe Thr	
	-10 -5 1	
15	AAC TGT GAC TTT GAG AAG ATT AAA GCA GCC TAT CTC AGT ACT ATT TCT	144
	Asn Cys Asp Phe Glu Lys Ile Lys Ala Ala Tyr Leu Ser Thr Ile Ser	
	5 10 15 20	
20	AAA GAC CTG ATT ACA TAT ATG AGT GGG ACC AAA AGT ACC GAG TTC AAC	192
	Lys Asp Leu Ile Thr Tyr Met Ser Gly Thr Lys Ser Thr Glu Phe Asn	
	25 30 35	
25	AAC ACC GTC TCT TGT AGC AAT CGG CCA CAT TGC CTT ACT GAA ATC CAG	240
	Asn Thr Val Ser Cys Ser Asn Arg Pro His Cys Leu Thr Glu Ile Gln	
	40 45 50	
30	AGC CTA ACC TTC AAT CCC ACC GCC GGC TGC GCG TCG CTC GCC AAA GAA	288
	Ser Leu Thr Phe Asn Pro Thr Ala Gly Cys Ala Ser Leu Ala Lys Glu	
	55 60 65	
35	ATG TTC GCC ATG AAA ACT AAG GCT GCC TTA GCT ATC TGG TGC CCA GGC	336
	Met Phe Ala Met Lys Thr Lys Ala Ala Leu Ala Ile Trp Cys Pro Gly	
	70 75 80	
40	TAT TCG GAA ACT CAG ATA AAT GCT ACT CAG GCA ATG AAG AAG AGG AGA	384
	Tyr Ser Glu Thr Gln Ile Asn Ala Thr Gln Ala Met Lys Lys Arg Arg	
	85 90 95 100	
45	AAA AGG AAA GTC ACA ACC AAT AAA TGT CTG GAA CAA GTG TCA CAA TTA	432
	Lys Arg Lys Val Thr Thr Asn Lys Cys Leu Glu Gln Val Ser Gln Leu	
	105 110 115	
50	CAA GGA TTG TGG CGT CGC TTC AAT CGA CCT TTA CTG AAA CAA CAG	477
	Gln Gly Leu Trp Arg Arg Phe Asn Arg Pro Leu Leu Lys Gln Gln	
	120 125 130	
55	TAA	480

MFPFALLYVLSVSFRKIFILQLVGLVLT

YDFTNCDFEKIKAAYLSTISKDLITYMSGTKSTEFNNTVSCSNRPHCLTEIQSLTFNPTAGCASLAKEM

FAMKTKAALAIWCPGYSETQINATQAMKKRRKRKVTNNKCLEQVSQLQLWRRFNRPLLKQQ

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Table 2: Comparison of various IL-7 embodiments compared to IL-B50. The IL-7 from sheep is SEQ ID NO: 5; from bovine is SEQ ID NO: 6; from human is SEQ ID NO: 7; from mouse is SEQ ID NO: 8; and from rat is SEQ ID NO: 9. See also GenBank.

5		**	**** *	***	** * *	* *
	IL-7human	MFH.....	.VSFRYIFGL	PPLILVLLPV	ASSDCDIEGK	DGKQYESVLM
	IL-7sheep	MFH.....	.VSFRYIFGI	PPLILVLLPV	ASSDCDFSGK	DGGAYQNVLM
	IL-7bovin	MFH.....	.VSFRYIFGI	PPLILVLLPV	ASSDCDISGK	DGGAYQNVLM
10	IL-7mouse	MFH.....	.VSFRYIFGI	PPLILVLLPV	TSSECHIKDK	EGKAYESVLM
	IL-7rat	MFH.....	.VSFRYIFGI	PPLILVLLPV	TSSDCHIKDK	DGKAFGSVLM
	ILB50human	MFPFALLYVL	SVSFRKIFIL	QLVGLVLT.Y	DFTNCDFE.K	IKAAAYLSTIS

						helix A
15			* * *	* *		
	IL-7human	VSIDQLLDSM	KEIGSNCLNN	EFNFFKRHIC	DANKEGMFLF	RAARKLRQFL
	IL-7sheep	VSIDDL.DNM	INFDSNCLNN	EPNFFKKHSC	DDNKEASFLN	RAARKLKQFL
	IL-7bovin	VNIDDL.DNM	INFDSNCLNN	EPNFFKKHSC	DDNKEASFLN	RASRKLRQFL
20	IL-7mouse	ISIDEL.DKM	TGTDSCNCPNN	EPNFFKRHVC	DDTKEAAFLN	RAARKLKQFL
	IL-7rat	ISINQL.DKM	TGTDSDCPNN	EPNFFKKHLC	DDTKEAAFLN	RAARKLRQFL
	ILB50human	KDLITY...M	SGTKSTEFNN	TVSCSNRPHC	LTEIQSLTFN	PTAGCASLAK
		-----		^^^^	-----	
				strand 1	helix B	
25			*	* *		*
	IL-7human	KMNSTGDFDL	HLLKVSEGTT	ILLNCTGQVK	GRKPAALGEA	QPTKSLEENK
	IL-7sheep	KMNISDDFKL	HLSTVSQGT	TLLNCTSKGK	GRKPPSLGEA	QPTKNLEENK
	IL-7bovin	KMNISDDFKL	HLSTVSQGT	TLLNCTSKGK	GRKPPSLSEA	QPTKNLEENK
30	IL-7mouse	KMNISEEFNV	HLLTVSQGTQ	TLVNCTSK..	EEK
	IL-7rat	KMNISEEFND	HLLRVSDGTQ	TLVNCTSK..	EEK
	ILB50human	EMFAMKTKAA	LAIWCPCGYSE	TQINATQA..	MK

			helix C			
35			*	*		
	IL-7human	SLKEQKKLND	LCFLKRLLE	IKTCWNKILM	GTKEH	
	IL-7sheep	SLKEQRKQND	LCFLKILLQK	IKTCWNKILR	GITEH	
	IL-7bovin	SSKEQKKQND	LCFLKILLQK	IKTCWNKILR	GIKEH	
40	IL-7mouse	NVKEQKK.ND	ACFLKRLLE	IKTCWNKILK	GSI..	
	IL-7rat	TIKEQKK.ND	PCFLKRLLE	IKTCWNKILK	GSI..	
	ILB50human	KRRKRKVTTN	KCLEQVSQLO	GLWRRFNRPL	LKQQ.	
		-----	^^^^	-----		
			strand 2	helix D		

C in bold, predicted glycosylated N underlined, using second ATG for IL-B50, *=id with human IL-7.

Comparison of the sequences will also provide an evolutionary tree. This can be generated, e.g., using the TreeView program in combination with the ClustalX analysis software program. See Thompson, et al. Nuc. Acids Res. 25:4876-4882; and TreeView, Page, IBLs, University of Glasgow, e-mail rpage@bio.gla.ac.uk; <http://taxonomy.zoology.gla.ac.uk.rod.treeview.html>.

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The structural homology of IL-B50 to related cytokine proteins suggests related function of this molecule. IL-B50 is a short chain cytokine exhibiting sequence similarity to IL-7.

- 5 Many aspects of the biology of IL-7 is well
recognized. See, e.g., Bauer, et al. (1998) "Modulated
expression of the epidermal growth factor-like homeotic
protein dlk influences stromal-cell-pre-B-cell interactions,
stromal cell adipogenesis, and pre-B-cell interleukin-7
10 requirements" Mol. Cell. Biol. 18:5247-5255; Maeurer, et al.
(1998) "Interleukin-7 (IL-7) knockout mice. Implications for
lymphopoiesis and organ-specific immunity" Int. Rev.
Immunol. 16309-322; Mertsching, et al. (1998) "Interleukin-
7, a non-redundant potent cytokine whose over-expression
15 massively perturbs B-lymphopoiesis" Int. Rev. Immunol.
16:285-308; Maini, et al. (1997) "New developments in the
use of cytokines for cancer therapy" Anticancer Res.
17:3803-3808; Murray (1996) "Physiologic roles of
interleukin-2, interleukin-4, and interleukin-7" Curr. Opin.
20 Hematol. 3:230-234; Takatsu (1997) "Cytokines involved in B-
cell differentiation and their sites of action" Proc. Soc.
Exp. Biol. Med. 215:121-133; Candeias, et al. (1997) "IL-7
receptor and VDJ recombination: trophic versus mechanistic
actions" Immunity 6:501-508; Lachman, et al. (1996)
25 "Cytokine-containing liposomes as vaccine adjuvants" Eur.
Cytokine Netw. 7:693-698; Takashima, et al. (1996)
"Cytokine-mediated communication by keratinocytes and
Langerhans cells with dendritic epidermal T cells" Semin.
Immunol. 8:333-339; and Johnston, et al. (1996) "Signaling
30 by IL-2 and related cytokines: JAKs, STATs, and relationship
to immunodeficiency" J. Leukoc. Biol. 60:441-452. The
biology of the IL-B50 is likely to be similar. See, e.g.,
Friend, et al. (1994) Exp. Hematol. 22:321-328.

- IL-B50 agonists, or antagonists, may also act as
35 functional or receptor antagonists, e.g., which block IL-7
binding to its respective receptors, or mediating the

opposite actions. Thus, IL-B50, or its antagonists, may be useful in the treatment of abnormal medical conditions, including immune disorders, e.g., T cell immune deficiencies, chronic inflammation, or tissue rejection, or in cardiovascular or neurophysiological conditions. Compositions combining the IL-B50 and IL-7 related reagents will often be used.

The natural antigens are capable of mediating various biochemical responses which lead to biological or physiological responses in target cells. The preferred embodiment characterized herein is from human, but other primate, or other species counterparts exist in nature. Additional sequences for proteins in other mammalian species, e.g., primates, canines, felines, and rodents, should also be available. See below. The descriptions below are directed, for exemplary purposes, to a human IL-B50, but are likewise applicable to related embodiments from other species.

II. Purified IL-B50

Primate, e.g., human, IL-B50 amino acid sequence, is shown as one embodiment within SEQ ID NO: 2 or 4. Other naturally occurring nucleic acids which encode the protein can be isolated by standard procedures using the provided sequence, e.g., PCR techniques, or by hybridization. These amino acid sequences, provided amino to carboxy, are important in providing sequence information for the cytokine allowing for distinguishing the protein antigen from other proteins and exemplifying numerous variants. Moreover, the peptide sequences allow preparation of peptides to generate antibodies to recognize such segments, and nucleotide sequences allow preparation of oligonucleotide probes, both of which are strategies for detection or isolation, e.g., cloning, of genes encoding such sequences.

As used herein, the term "human soluble IL-B50" shall encompass, when used in a protein context, a protein having amino acid sequence corresponding to a soluble polypeptide shown in SEQ ID NO: 2 or 4, or significant fragments thereof. Preferred embodiments comprise a plurality of distinct, e.g., nonoverlapping, segments of the specified length. Typically, the plurality will be at least two, more usually at least three, and preferably 5, 7, or even more. While the length minima are provided, longer lengths, of various sizes, may be appropriate, e.g., one of length 7, and two of length 12.

Binding components, e.g., antibodies, typically bind to an IL-B50 with high affinity, e.g., at least about 100 nM, usually better than about 30 nM, preferably better than about 10 nM, and more preferably at better than about 3 nM. Counterpart proteins will be found in mammalian species other than human, e.g., other primates, ungulates, or rodents. Non-mammalian species should also possess structurally or functionally related genes and proteins, e.g., birds or amphibians.

The term "polypeptide" as used herein includes a significant fragment or segment, and encompasses a stretch of amino acid residues of at least about 8 amino acids, generally at least about 12 amino acids, typically at least about 16 amino acids, preferably at least about 20 amino acids, and, in particularly preferred embodiments, at least about 30 or more amino acids, e.g., 35, 40, 45, 50, etc. Such fragments may have ends which begin and/or end at virtually all positions, e.g., beginning at residues 1, 2, 3, etc., and ending at, e.g., 150, 149, 148, etc., in all practical combinations. Particularly interesting peptides have ends corresponding to structural domain boundaries, e.g., helices A, B, C, and/or D. See Tables 1 and 2.

The term "binding composition" refers to molecules that bind with specificity to IL-B50, e.g., in an antibody-antigen interaction. The specificity may be more or less

inclusive, e.g., specific to a particular embodiment, or to groups of related embodiments, e.g., primate, rodent, etc.

It also includes compounds, e.g., proteins, which

specifically associate with IL-B50, including in a natural

5 physiologically relevant protein-protein interaction, either covalent or non-covalent. The molecule may be a polymer, or chemical reagent. A functional analog may be a protein with structural modifications, or it may be a molecule which has a molecular shape which interacts with the appropriate

10 binding determinants. The compounds may serve as agonists or antagonists of a receptor binding interaction, see, e.g., Goodman, et al. (eds.) Goodman & Gilman's: The Pharmacological Bases of Therapeutics (current ed.) Pergamon Press.

15 Substantially pure, e.g., in a protein context,
typically means that the protein is free from other
contaminating proteins, nucleic acids, or other biologicals
derived from the original source organism. Purity may be
assayed by standard methods, typically by weight, and will
20 ordinarily be at least about 40% pure, generally at least
about 50% pure, often at least about 60% pure, typically at
least about 80% pure, preferably at least about 90% pure,
and in most preferred embodiments, at least about 95% pure.
Carriers or excipients will often be added.

Solubility of a polypeptide or fragment depends upon the environment and the polypeptide. Many parameters affect polypeptide solubility, including temperature, electrolyte environment, size and molecular characteristics of the polypeptide, and nature of the solvent. Typically, the temperature at which the polypeptide is used ranges from about 4° C to about 65° C. Usually the temperature at use is greater than about 18° C. For diagnostic purposes, the temperature will usually be about room temperature or warmer, but less than the denaturation temperature of components in the assay. For therapeutic purposes, the temperature will usually be body temperature, typically

about 37° C for humans and mice, though under certain situations the temperature may be raised or lowered in situ or in vitro.

The size and structure of the polypeptide should generally be in a substantially stable state, and usually not in a denatured state. The polypeptide may be associated with other polypeptides in a quaternary structure, e.g., to confer solubility, or associated with lipids or detergents.

The solvent and electrolytes will usually be a biologically compatible buffer, of a type used for preservation of biological activities, and will usually approximate a physiological aqueous solvent. Usually the solvent will have a neutral pH, typically between about 5 and 10, and preferably about 7.5. On some occasions, one or more detergents will be added, typically a mild non-denaturing one, e.g., CHS (cholesteryl hemisuccinate) or CHAPS (3-[3-cholamidopropyl]dimethylammonio]-1-propane sulfonate), or a low enough concentration as to avoid significant disruption of structural or physiological properties of the protein. In other instances, a harsh detergent may be used to effect significant denaturation.

III. Physical Variants

This invention also encompasses proteins or peptides having substantial amino acid sequence identity with the amino acid sequence of the IL-B50 antigen. The variants include species, polymorphic, or allelic variants.

Amino acid sequence homology, or sequence identity, is determined by optimizing residue matches, if necessary, by introducing gaps as required. See also Needleham, et al. (1970) J. Mol. Biol. 48:443-453; Sankoff, et al. (1983) Chapter One in Time Warps, String Edits, and Macromolecules: The Theory and Practice of Sequence Comparison, Addison-Wesley, Reading, MA; and software packages from IntelliGenetics, Mountain View, CA; and the University of Wisconsin Genetics Computer Group, Madison, WI. Sequence

identity changes when considering conservative substitutions as matches. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. The conservation may apply to biological features, functional features, or structural features. Homologous amino acid sequences are typically intended to include natural polymorphic or allelic and interspecies variations of a protein sequence. Typical homologous proteins or peptides will have from 25-100% identity (if gaps can be introduced), to 50-100% identity (if conservative substitutions are included) with the amino acid sequence of the IL-B50.

Identity measures will be at least about 35%, generally at least about 40%, often at least about 50%, typically at least about 60%, usually at least about 70%, preferably at least about 80%, and more preferably at least about 90%.

The isolated IL-B50 DNA can be readily modified by nucleotide substitutions, nucleotide deletions, nucleotide insertions, and inversions of short nucleotide stretches. These modifications result in novel DNA sequences which encode these antigens, their derivatives, or proteins having similar physiological, immunogenic, antigenic, or other functional activity. These modified sequences can be used to produce mutant antigens or to enhance expression. Enhanced expression may involve gene amplification, increased transcription, increased translation, and other mechanisms. "Mutant IL-B50" encompasses a polypeptide otherwise falling within the sequence identity definition of the IL-B50 as set forth above, but having an amino acid sequence which differs from that of IL-B50 as normally found in nature, whether by way of deletion, substitution, or insertion. This generally includes proteins having significant identity with a protein having sequence of SEQ ID NO: 2 or 4, and as sharing various biological activities,

IL-B50 mutagenesis can also be conducted by making amino acid insertions or deletions. Substitutions, deletions, insertions, or any combinations may be generated to arrive at a final construct. Insertions include amino- or carboxy- terminal fusions. Random mutagenesis can be conducted at a target codon and the expressed mutants can then be screened for the desired activity. Methods for making substitution mutations at predetermined sites in DNA having a known sequence are well known in the art, e.g., by M13 primer mutagenesis or polymerase chain reaction (PCR) techniques. See, e.g., Sambrook, et al. (1989); Ausubel, et al. (1987 and Supplements); and Kunkel, et al. (1987) Methods in Enzymol. 154:367-382. Preferred embodiments include, e.g., 1-fold, 2-fold, 3-fold, 5-fold, 7-fold, etc., preferably conservative substitutions at the nucleotide or amino acid levels. Preferably the substitutions will be away from the conserved cysteines, and often will be in the regions away from the helical structural domains. Such variants may be useful to produce specific antibodies, and often will share many or all biological properties.

The present invention also provides recombinant proteins, e.g., heterologous fusion proteins using segments from these proteins. A heterologous fusion protein is a fusion of proteins or segments which are naturally not

normally fused in the same manner. A similar concept applies to heterologous nucleic acid sequences.

In addition, new constructs may be made from combining similar functional domains from other proteins. For
5 example, target-binding or other segments may be "swapped" between different new fusion polypeptides or fragments. See, e.g., Cunningham, et al. (1989) Science 243:1330-1336; and O'Dowd, et al. (1988) J. Biol. Chem. 263:15985-15992.

The phosphoramidite method described by Beaucage and
10 Carruthers (1981) Tetra. Letts. 22:1859-1862, will produce suitable synthetic DNA fragments. A double stranded fragment will often be obtained either by synthesizing the complementary strand and annealing the strand together under appropriate conditions or by adding the complementary strand
15 using DNA polymerase with an appropriate primer sequence, e.g., PCR techniques.

Structural analysis can be applied to this gene, in comparison to the IL-7 family of cytokines. Alignment of the human IL-B50 sequences with other members of the IL-7
20 family should allow definition of structural features. In particular, β -sheet and α -helix residues can be determined using, e.g., RASMOL program, see Bazan, et al. (1996) Nature 379:591; Lodi, et al. (1994) Science 263:1762-1766; Sayle and Milner-White (1995) TIBS 20:374-376; and Gronenberg, et
25 al. (1991) Protein Engineering 4:263-269. Preferred residues for substitutions include the surface exposed residues which would be predicted to interact with receptor. Other residues which should conserve function will be conservative substitutions, particularly at position far
30 from the surface exposed residues.

IV. Functional Variants

The blocking of physiological response to IL-B50s may result from the competitive inhibition of binding of the
35 ligand to its receptor.

In vitro assays of the present invention will often use isolated protein, soluble fragments comprising receptor binding segments of these proteins, or fragments attached to solid phase substrates. These assays will also allow for the diagnostic determination of the effects of either binding segment mutations and modifications, or cytokine mutations and modifications, e.g., IL-B50 analogs.

This invention also contemplates the use of competitive drug screening assays, e.g., where neutralizing antibodies to the cytokine, or receptor binding fragments compete with a test compound.

"Derivatives" of IL-B50 antigens include amino acid sequence mutants from naturally occurring forms, glycosylation variants, and covalent or aggregate conjugates with other chemical moieties. Covalent derivatives can be prepared by linkage of functionalities to groups which are found in IL-B50 amino acid side chains or at the N- or C-termini, e.g., by standard means. See, e.g., Lundblad and Noyes (1988) Chemical Reagents for Protein Modification, vols. 1-2, CRC Press, Inc., Boca Raton, FL; Hugli (ed. 1989) Techniques in Protein Chemistry, Academic Press, San Diego, CA; and Wong (1991) Chemistry of Protein Conjugation and Cross Linking, CRC Press, Boca Raton, FL.

In particular, glycosylation alterations are included, e.g., made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing, or in further processing steps. See, e.g., Elbein (1987) Ann. Rev. Biochem. 56:497-534. Also embraced are versions of the peptides with the same primary amino acid sequence which have other minor modifications, including phosphorylated amino acid residues, e.g., phosphotyrosine, phosphoserine, or phosphothreonine.

Fusion polypeptides between IL-B50s and other homologous or heterologous proteins are also provided. Many cytokine receptors or other surface proteins are multimeric, e.g., homodimeric entities, and a repeat construct may have

various advantages, including lessened susceptibility to proteolytic cleavage. Typical examples are fusions of a reporter polypeptide, e.g., luciferase, with a segment or domain of a protein, e.g., a receptor-binding segment, so that the presence or location of the fused ligand may be easily determined. See, e.g., Dull, et al., U.S. Patent No. 4,859,609. Other gene fusion partners include bacterial β -galactosidase, trpE, Protein A, β -lactamase, alpha amylase, alcohol dehydrogenase, yeast alpha mating factor, and detection or purification tags such as a FLAG sequence of His6 sequence. See, e.g., Godowski, et al. (1988) Science 241:812-816.

Fusion peptides will typically be made by either recombinant nucleic acid methods or by synthetic polypeptide methods. Techniques for nucleic acid manipulation and expression are described generally, e.g., in Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual (2d ed.), vols. 1-3, Cold Spring Harbor Laboratory; and Ausubel, et al. (eds. 1993) Current Protocols in Molecular Biology, Greene and Wiley, NY. Techniques for synthesis of polypeptides are described, e.g., in Merrifield (1963) J. Amer. Chem. Soc. 85:2149-2156; Merrifield (1986) Science 232: 341-347; Atherton, et al. (1989) Solid Phase Peptide Synthesis: A Practical Approach, IRL Press, Oxford; and Grant (1992) Synthetic Peptides: A User's Guide, W.H. Freeman, NY. Refolding methods may be applicable to synthetic proteins.

This invention also contemplates the use of derivatives of IL-B50 proteins other than variations in amino acid sequence or glycosylation. Such derivatives may involve covalent or aggregative association with chemical moieties or protein carriers. Covalent or aggregative derivatives will be useful as immunogens, as reagents in immunoassays, or in purification methods such as for affinity purification of binding partners, e.g., other antigens. An IL-B50 can be immobilized by covalent bonding

to a solid support such as cyanogen bromide-activated SEPHAROSE, by methods which are well known in the art, or adsorbed onto polyolefin surfaces, with or without glutaraldehyde cross-linking, for use in the assay or purification of anti-IL-B50 antibodies or an alternative binding composition. The IL-B50 proteins can also be labeled with a detectable group, e.g., for use in diagnostic assays. Purification of IL-B50 may be effected by an immobilized antibody or complementary binding partner, e.g., binding portion of a receptor.

A solubilized IL-B50 or fragment of this invention can be used as an immunogen for the production of antisera or antibodies specific for binding. Purified antigen can be used to screen monoclonal antibodies or antigen-binding fragments, encompassing antigen binding fragments of natural antibodies, e.g., Fab, Fab', F(ab)₂, etc. Purified IL-B50 antigens can also be used as a reagent to detect antibodies generated in response to the presence of elevated levels of the cytokine, which may be diagnostic of an abnormal or specific physiological or disease condition. This invention contemplates antibodies raised against amino acid sequences encoded by nucleotide sequence shown in SEQ ID NO: 1 or 3, or fragments of proteins containing it. In particular, this invention contemplates antibodies having binding affinity to or being raised against specific domains, e.g., helices A, B, C, or D.

The present invention contemplates the isolation of additional closely related species variants. Southern and Northern blot analysis will establish that similar genetic entities exist in other mammals. It is likely that IL-B50s are widespread in species variants, e.g., rodents, lagomorphs, carnivores, artiodactyla, perissodactyla, and primates.

The invention also provides means to isolate a group
35 of related antigens displaying both distinctness and
similarities in structure, expression, and function.

5 invention provides useful probes for identifying additional homologous genetic entities in different species.

10 negative background activity. This should allow analysis of
the function of IL-B50 in comparison to untransformed
control cells.

15 these antigens is possible using standard techniques of
modern molecular biology, particularly in comparing members
of the related class. See, e.g., the homolog-scanning
mutagenesis technique described in Cunningham, et al. (1989)
Science 243:1339-1336; and approaches used in O'Dowd, et al.
20 (1988) J. Biol. Chem. 263:15985-15992; and Lechleiter, et
al. (1990) EMBO J. 9:4381-4390.

intracellular components and cytokine may occur. Specific segments of interaction of IL-B50 with interacting components may be identified by mutagenesis or direct biochemical means, e.g., cross-linking or affinity methods. Structural analysis by crystallographic or other physical methods will also be applicable. Further investigation of the mechanism of signal transduction will include study of associated components which may be isolatable by affinity methods or by genetic means, e.g., complementation analysis of mutants.

35 Further study of the expression and control of IL-B50
will be pursued. The controlling elements associated with

Case	Age	Sex	Occupation	Time of Onset	Time of Death	Time of Autopsy
1	20	M	Student	10:00	11:00	11:30
2	25	F	Teacher	12:00	13:00	13:30
3	30	M	Engineer	14:00	15:00	15:30
4	35	F	Housewife	16:00	17:00	17:30
5	40	M	Doctor	18:00	19:00	19:30
6	45	F	Nurse	20:00	21:00	21:30
7	50	M	Lawyer	22:00	23:00	23:30
8	55	F	Artist	24:00	25:00	25:30
9	60	M	Farmer	26:00	27:00	27:30
10	65	F	Retired	28:00	29:00	29:30

the antigens should exhibit differential physiological, developmental, tissue specific, or other expression patterns. Upstream or downstream genetic regions, e.g., control elements, are of interest.

- 5 Structural studies of the IL-B50 antigens will lead to design of new antigens, particularly analogs exhibiting agonist or antagonist properties on the molecule. This can be combined with previously described screening methods to isolate antigens exhibiting desired spectra of activities.

10

V. Antibodies

- Antibodies can be raised to various epitopes of the IL-B50 proteins, including species, polymorphic, or allelic variants, and fragments thereof, both in their naturally occurring forms and in their recombinant forms.
- 15 Additionally, antibodies can be raised to IL-B50s in either their active forms or in their inactive forms, including native or denatured versions. Anti-idiotypic antibodies are also contemplated.

- 20 Antibodies, including binding fragments and single chain versions, against predetermined fragments of the antigens can be raised by immunization of animals with conjugates of the fragments with immunogenic proteins. Monoclonal antibodies are prepared from cells secreting the
- 25 desired antibody. These antibodies can be screened for binding to normal or defective IL-B50s, or screened for agonistic or antagonistic activity, e.g., mediated through a receptor. Antibodies may be agonistic or antagonistic, e.g., by sterically blocking binding to a receptor. These
- 30 monoclonal antibodies will usually bind with at least a K_D of about 1 mM, more usually at least about 300 μ M, typically at least about 100 μ M, more typically at least about 30 μ M, preferably at least about 10 μ M, and more preferably at least about 3 μ M or better.

- 35 An IL-B50 protein that specifically binds to or that is specifically immunoreactive with an antibody generated

against a defined immunogen, such as an immunogen consisting of the amino acid sequence of SEQ ID NO: 2 or 4, is typically determined in an immunoassay. The immunoassay typically uses a polyclonal antiserum which was raised, e.g., to a protein of SEQ ID NO: 2 or 4. This antiserum is selected to have low crossreactivity against other IL-7, e.g., human or rodent IL-7, preferably from the same species, and any such crossreactivity is removed by immunoabsorption prior to use in the immunoassay.

10 In order to produce antisera for use in an immunoassay, the protein of SEQ ID NO: 2 or 4, or a combination thereof, is isolated as described herein. For example, recombinant protein may be produced in a mammalian cell line. An appropriate host, e.g., an inbred strain of mice such as Balb/c, is immunized with the selected protein, typically using a standard adjuvant, such as Freund's adjuvant, and a standard mouse immunization protocol (see Harlow and Lane, supra). Alternatively, a synthetic peptide derived from the sequences disclosed herein and conjugated to a carrier protein can be used as an immunogen. Polyclonal sera are collected and titered against the immunogen protein in an immunoassay, e.g., a solid phase immunoassay with the immunogen immobilized on a solid support. Polyclonal antisera with a titer of 10^4 or greater are selected and tested for their cross reactivity against other IL-7 family members, e.g., rodent IL-7, using a competitive binding immunoassay such as the one described in Harlow and Lane, supra, at pages 570-573. Preferably at least one other IL-7 family member is used in this determination in conjunction with, e.g., the primate IL-7. The IL-7 family members can be produced as recombinant proteins and isolated using standard molecular biology and protein chemistry techniques as described herein.

Immunoassays in the competitive binding format can be used for the crossreactivity determinations. For example, the protein of SEQ ID NO: 4 can be immobilized to a solid

support. Proteins added to the assay compete with the binding of the antisera to the immobilized antigen. The ability of the above proteins to compete with the binding of the antisera to the immobilized protein is compared to the protein of SEQ ID NO: 4. The percent crossreactivity for the above proteins is calculated, using standard calculations. Those antisera with less than 10% crossreactivity with each of the proteins listed above are selected and pooled. The cross-reacting antibodies are then removed from the pooled antisera by immunoabsorption with the above-listed proteins.

The immunoabsorbed and pooled antisera are then used in a competitive binding immunoassay as described above to compare a second protein to the immunogen protein (e.g., the IL-7 like protein of SEQ ID NO: 2 or 4). In order to make this comparison, the two proteins are each assayed at a wide range of concentrations and the amount of each protein required to inhibit 50% of the binding of the antisera to the immobilized protein is determined. If the amount of the second protein required is less than twice the amount of the protein of the selected protein or proteins that is required, then the second protein is said to specifically bind to an antibody generated to the immunogen.

The antibodies of this invention can also be useful in diagnostic applications. As capture or non-neutralizing antibodies, they can be screened for ability to bind to the antigens without inhibiting binding to a receptor. As neutralizing antibodies, they can be useful in competitive binding assays. They will also be useful in detecting or quantifying IL-B50 protein or its receptors. See, e.g., Chan (ed. 1987) Immunology: A Practical Guide, Academic Press, Orlando, FL; Price and Newman (eds. 1991) Principles and Practice of Immunoassay, Stockton Press, N.Y.; and Ngo (ed. 1988) Nonisotopic Immunoassay, Plenum Press, N.Y.

Cross absorptions, depletions, or other means will provide preparations of defined selectivity, e.g., unique or shared

species specificities. These may be the basis for tests which will identify various groups of antigens.

Further, the antibodies, including antigen binding fragments, of this invention can be potent antagonists that bind to the antigen and inhibit functional binding, e.g., to a receptor which may elicit a biological response. They also can be useful as non-neutralizing antibodies and can be coupled to toxins or radionuclides so that when the antibody binds to antigen, a cell expressing it, e.g., on its surface, is killed. Further, these antibodies can be conjugated to drugs or other therapeutic agents, either directly or indirectly by means of a linker, and may effect drug targeting.

Antigen fragments may be joined to other materials, particularly polypeptides, as fused or covalently joined polypeptides to be used as immunogens. An antigen and its fragments may be fused or covalently linked to a variety of immunogens, such as keyhole limpet hemocyanin, bovine serum albumin, tetanus toxoid, etc. See Microbiology, Hoeber Medical Division, Harper and Row, 1969; Landsteiner (1962) Specificity of Serological Reactions, Dover Publications, New York; Williams, et al. (1967) Methods in Immunology and Immunochemistry, vol. 1, Academic Press, New York; and Harlow and Lane (1988) Antibodies: A Laboratory Manual, CSH Press, NY, for descriptions of methods of preparing polyclonal antisera.

In some instances, it is desirable to prepare monoclonal antibodies from various mammalian hosts, such as mice, rodents, primates, humans, etc. Description of techniques for preparing such monoclonal antibodies may be found in, e.g., Stites, et al. (eds.) Basic and Clinical Immunology (4th ed.), Lange Medical Publications, Los Altos, CA, and references cited therein; Harlow and Lane (1988) Antibodies: A Laboratory Manual, CSH Press; Goding (1986) Monoclonal Antibodies: Principles and Practice (2d ed.), Academic Press, New York; and particularly in Kohler and

Milstein (1975) in Nature 256:495-497, which discusses one method of generating monoclonal antibodies.

Other suitable techniques involve in vitro exposure of lymphocytes to the antigenic polypeptides or alternatively to selection of libraries of antibodies in phage or similar vectors. See, Huse, et al. (1989) "Generation of a Large Combinatorial Library of the Immunoglobulin Repertoire in Phage Lambda," Science 246:1275-1281; and Ward, et al. (1989) Nature 341:544-546. The polypeptides and antibodies of the present invention may be used with or without modification, including chimeric or humanized antibodies. Frequently, the polypeptides and antibodies will be labeled by joining, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, chemiluminescent moieties, magnetic particles, and the like. Patents, teaching the use of such labels include U.S. Patent Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241. Also, recombinant or chimeric immunoglobulins may be produced, see Cabilly, U.S. Patent No. 4,816,567; Moore, et al., U.S. Patent No. 4,642,334; and Queen, et al. (1989) Proc. Nat'l Acad. Sci. USA 86:10029-10033; or made in transgenic mice, see Mendez, et al. (1997) Nature Genetics 15:146-156. These references are incorporated herein by reference.

The antibodies of this invention can also be used for affinity chromatography in isolating the protein. Columns can be prepared where the antibodies are linked to a solid support. See, e.g., Wilchek et al. (1984) Meth. Enzymol. 104:3-55. The converse may be used to purify antibodies.

Antibodies raised against each IL-B50 will also be useful to raise anti-idiotypic antibodies. These will be

VI. Nucleic Acids

The peptide segments can also be used to predict appropriate oligonucleotides to screen a library. The genetic code can be used to select appropriate oligonucleotides useful as probes for screening. See, e.g., SEQ ID NO: 1 or 3. In combination with polymerase chain reaction (PCR) techniques, synthetic oligonucleotides will

be useful in selecting correct clones from a library. Complementary sequences will also be used as probes, primers, or antisense strands. Various fragments should be particularly useful, e.g., coupled with anchored vector or poly-A complementary PCR techniques or with complementary DNA of other peptides.

This invention contemplates use of isolated DNA or fragments to encode a biologically active corresponding IL-B50 polypeptide, particularly lacking the portion coding the untranslated 5' portion of the described sequence. In addition, this invention covers isolated or recombinant DNA which encodes a biologically active protein or polypeptide and which is capable of hybridizing under appropriate conditions with the DNA sequences described herein. Said biologically active protein or polypeptide can be an intact antigen, or fragment, and have an amino acid sequence disclosed in, e.g., SEQ ID NO: 2 or 4, particularly a mature, secreted polypeptide. Further, this invention covers the use of isolated or recombinant DNA, or fragments thereof, which encode proteins which exhibit high identity to a secreted IL-B50. The isolated DNA can have the respective regulatory sequences in the 5' and 3' flanks, e.g., promoters, enhancers, poly-A addition signals, and others. Alternatively, expression may be effected by operably linking a coding segment to a heterologous promoter, e.g., by inserting a promoter upstream from an endogenous gene. See, e.g., Treco, et al. WO96/29411 or USSN 08/406,030.

An "isolated" nucleic acid is a nucleic acid, e.g., an RNA, DNA, or a mixed polymer, which is substantially separated from other components which naturally accompany a native sequence, e.g., ribosomes, polymerases, and/or flanking genomic sequences from the originating species. The term embraces a nucleic acid sequence which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically

synthesized analogs or analogs biologically synthesized by heterologous systems. A substantially pure molecule includes isolated forms of the molecule. Generally, the nucleic acid will be in a vector or fragment less than about 50 kb, usually less than about 30 kb, typically less than about 10 kb, and preferably less than about 6 kb.

An isolated nucleic acid will generally be a homogeneous composition of molecules, but will, in some embodiments, contain minor heterogeneity. This heterogeneity is typically found at the polymer ends or portions not critical to a desired biological function or activity.

A "recombinant" nucleic acid is defined either by its method of production or its structure. In reference to its method of production, e.g., a product made by a process, the process is use of recombinant nucleic acid techniques, e.g., involving human intervention in the nucleotide sequence, typically selection or production. Alternatively, it can be a nucleic acid made by generating a sequence comprising fusion of two fragments which are not naturally contiguous to each other, but is meant to exclude products of nature, e.g., naturally occurring mutants. Thus, e.g., products made by transforming cells with any unnaturally occurring vector is encompassed, as are nucleic acids comprising sequence derived using any synthetic oligonucleotide process. Such is often done to replace a codon with a redundant codon encoding the same or a conservative amino acid, while typically introducing or removing a sequence recognition site.

Alternatively, it is performed to join together nucleic acid segments of desired functions to generate a single genetic entity comprising a desired combination of functions not found in the commonly available natural forms. Restriction enzyme recognition sites are often the target of such artificial manipulations, but other site specific targets, e.g., promoters, DNA replication sites, regulation

Recombinant clones derived from the genomic sequences,
30 e.g., containing introns, will be useful for transgenic
studies, including, e.g., transgenic cells and organisms,
and for gene therapy. See, e.g., Goodnow (1992) "Transgenic
Animals" in Roitt (ed.) Encyclopedia of Immunology, Academic
Press, San Diego, pp. 1502-1504; Travis (1992) Science
35 256:1392-1394; Kuhn, et al. (1991) Science 254:707-710;
Capeocchi (1989) Science 244:1288; Robertson (ed. 1987)

Teratocarcinomas and Embryonic Stem Cells: A Practical Approach, IRL Press, Oxford; and Rosenberg (1992) J. Clinical Oncology 10:180-199.

Substantial homology, e.g., identity, in the nucleic acid sequence comparison context means either that the segments, or their complementary strands, when compared, are identical when optimally aligned, with appropriate nucleotide insertions or deletions, in at least about 50% of the nucleotides, generally at least about 58%, ordinarily at least about 65%, often at least about 71%, typically at least about 77%, usually at least about 85%, preferably at least about 95 to 98% or more, and in particular embodiments, as high as about 99% or more of the nucleotides. Alternatively, substantial homology exists when the segments will hybridize under selective hybridization conditions, to a strand, or its complement, typically using a sequence of IL-B50, e.g., in SEQ ID NO: 1 or 3. Typically, selective hybridization will occur when there is at least about 55% identity over a stretch of at least about 30 nucleotides, preferably at least about 75% over a stretch of about 25 nucleotides, and most preferably at least about 90% over about 20 nucleotides. See, Kanehisa (1984) Nuc. Acids Res. 12:203-213. The length of identity comparison, as described, may be over longer stretches, and in certain embodiments will be over a stretch of at least about 17 nucleotides, usually at least about 28 nucleotides, typically at least about 40 nucleotides, and preferably at least about 75 to 100 or more nucleotides.

Stringent conditions, in referring to homology in the hybridization context, will be stringent combined conditions of salt, temperature, organic solvents, and other parameters, typically those controlled in hybridization reactions. Stringent temperature conditions will usually include temperatures in excess of about 30° C, usually in excess of about 37° C, typically in excess of about 55° C, 60° C, 65° C, or preferably in excess of about 70° C.

Stringent salt conditions will ordinarily be less than about 1000 or 600 mM usually less than about 400 mM, typically less than about 250 mM, preferably less than about 150 mM, including about 100, 50, or even 20 mM. However, the combination of parameters is much more important than the measure of any single parameter. See, e.g., Wetmur and Davidson (1968) J. Mol. Biol. 31:349-370. Hybridization under stringent conditions should give a background of at least 2-fold over background, preferably at least 3-5 or more.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, 15 subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program 20 parameters.

Optical alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith and Waterman (1981) Adv. Appl. Math. 2:482, by the homology alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48:443, by the search for similarity method of Pearson and Lipman (1988) Proc. Nat'l Acad. Sci. USA 85:2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection (see generally Ausubel et al., supra).

One example of a useful algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments to show relationship and percent sequence identity. It also plots a tree or dendrogram showing the clustering

relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng and Doolittle (1987) J. Mol. Evol. 35:351-360. The method used is similar to the method described by Higgins and Sharp (1989) CABIOS 5:151-153. The program can align up to 300 sequences, each of a maximum length of 5,000 nucleotides or amino acids. The multiple alignment procedure begins with the pairwise alignment of the two most similar sequences, producing a cluster of two aligned sequences. This cluster is then aligned to the next most related sequence or cluster of aligned sequences. Two clusters of sequences are aligned by a simple extension of the pairwise alignment of two individual sequences. The final alignment is achieved by a series of progressive, pairwise alignments. The program is run by designating specific sequences and their amino acid or nucleotide coordinates for regions of sequence comparison and by designating the program parameters. For example, a reference sequence can be compared to other test sequences to determine the percent sequence identity relationship using the following parameters: default gap weight (3.00), default gap length weight (0.10), and weighted end gaps.

Another example of algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described Altschul, et al. (1990) J. Mol. Biol. 215:403-410. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul, et al., supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits

are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a wordlength (W) of 11, the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1989) Proc. Nat'l Acad. Sci. USA 89:10915) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin and Altschul (1993) Proc. Nat'l Acad. Sci. USA 90:5873-5787). One measure of similarity provided by the BLAST algorithm is the smallest sum probability ($P(N)$), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

A further indication that two nucleic acid sequences of polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that

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the two molecules hybridize to each other under stringent conditions, as described below.

IL-B50 from other mammalian species can be cloned and isolated by cross-species hybridization of closely related species. Homology may be relatively low between distantly related species, and thus hybridization of relatively closely related species is advisable. Alternatively, preparation of an antibody preparation which exhibits less species specificity may be useful in expression cloning approaches.

VII. Making IL-B50; Mimetics

DNA which encodes the IL-B50 or fragments thereof can be obtained by chemical synthesis, screening cDNA libraries, or screening genomic libraries prepared from a wide variety of cell lines or tissue samples. See, e.g., Okayama and Berg (1982) Mol. Cell. Biol. 2:161-170; Gubler and Hoffman (1983) Gene 25:263-269; and Glover (ed. 1984) DNA Cloning: A Practical Approach, IRL Press, Oxford. Alternatively, the sequences provided herein provide useful PCR primers or allow synthetic or other preparation of suitable genes encoding an IL-B50; including naturally occurring embodiments.

This DNA can be expressed in a wide variety of host cells for the synthesis of a full-length IL-B50 or fragments which can in turn, e.g., be used to generate polyclonal or monoclonal antibodies; for binding studies; for construction and expression of modified molecules; and for structure/function studies.

Vectors, as used herein, comprise plasmids, viruses, bacteriophage, integratable DNA fragments, and other vehicles which enable the integration of DNA fragments into the genome of the host. See, e.g., Pouwels, et al. (1985 and Supplements) Cloning Vectors: A Laboratory Manual, Elsevier, N.Y.; and Rodriguez, et al. (eds. 1988) Vectors:

A Survey of Molecular Cloning Vectors and Their Uses,
Buttersworth, Boston, MA.

For purposes of this invention, DNA sequences are operably linked when they are functionally related to each other. For example, DNA for a presequence or secretory leader is operably linked to a polypeptide if it is expressed as a preprotein or participates in directing the polypeptide to the cell membrane or in secretion of the polypeptide. A promoter is operably linked to a coding sequence if it controls the transcription of the polypeptide; a ribosome binding site is operably linked to a coding sequence if it is positioned to permit translation. Usually, operably linked means contiguous and in reading frame, however, certain genetic elements such as repressor genes are not contiguously linked but still bind to operator sequences that in turn control expression. See, e.g., Rodriguez, et al., Chapter 10, pp. 205-236; Balbas and Bolivar (1990) Methods in Enzymology 185:14-37; and Ausubel, et al. (1993) Current Protocols in Molecular Biology, Greene and Wiley, NY.

Representative examples of suitable expression vectors include pCDNA1; pCD, see Okayama, et al. (1985) Mol. Cell Biol. 5:1136-1142; pMC1neo Poly-A, see Thomas, et al. (1987) Cell 51:503-512; and a baculovirus vector such as pAC 373 or pAC 610. See, e.g., Miller (1988) Ann. Rev. Microbiol. 42:177-199.

It will often be desired to express an IL-B50 polypeptide in a system which provides a specific or defined glycosylation pattern. See, e.g., Luckow and Summers (1988) Bio/Technology 6:47-55; and Kaufman (1990) Meth. Enzymol. 185:487-511.

The IL-B50, or a fragment thereof, may be engineered to be phosphatidyl inositol (PI) linked to a cell membrane, but can be removed from membranes by treatment with a phosphatidyl inositol cleaving enzyme, e.g., phosphatidyl inositol phospholipase-C. This releases the antigen in a

biologically active form, and allows purification by standard procedures of protein chemistry. See, e.g., Low (1989) Biochim. Biophys. Acta 988:427-454; Tse, et al. (1985) Science 230:1003-1008; and Brunner, et al. (1991) J. Cell Biol. 114:1275-1283.

Now that the IL-B50 has been characterized, fragments or derivatives thereof can be prepared by conventional processes for synthesizing peptides. These include processes such as are described in Stewart and Young (1984) Solid Phase Peptide Synthesis, Pierce Chemical Co., Rockford, IL; Bodanszky and Bodanszky (1984) The Practice of Peptide Synthesis, Springer-Verlag, New York; Bodanszky (1984) The Principles of Peptide Synthesis, Springer-Verlag, New York; and Villafranca (ed. 1991) Techniques in Protein Chemistry II, Academic Press, San Diego, Ca.

VIII. Uses

The present invention provides reagents which will find use in diagnostic applications as described elsewhere herein, e.g., in IL-B50 mediated conditions, or below in the description of kits for diagnosis. The gene may be useful in forensic sciences, e.g., to distinguish rodent from human, or as a marker to distinguish between different cells exhibiting differential expression or modification patterns.

This invention also provides reagents with significant commercial and/or therapeutic potential. The IL-B50 (naturally occurring or recombinant), fragments thereof, and antibodies thereto, along with compounds identified as having binding affinity to IL-B50, should be useful as reagents for teaching techniques of molecular biology, immunology, or physiology. Appropriate kits may be prepared with the reagents, e.g., in practical laboratory exercises in production or use of proteins, antibodies, cloning methods, histology, etc.

The reagents will also be useful in the treatment of conditions associated with abnormal physiology or

development, including inflammatory conditions. They may be useful in vitro tests for presence or absence of interacting components, which may correlate with success of particular treatment strategies. In particular, modulation of

5 physiology of various, e.g., hematopoietic or lymphoid, cells will be achieved by appropriate methods for treatment using the compositions provided herein. See, e.g., Thomson (ed. 1998) The Cytokine Handbook (3d ed.) Academic Press, San Diego; Metcalf and Nicola (1995) The Hematopoietic

10 Colony Stimulating Factors Cambridge University Press; and Aggarwal and Gutterman (1991) Human Cytokines Blackwell Pub.

For example, a disease or disorder associated with abnormal expression or abnormal signaling by an IL-B50 should be a likely target for an agonist or antagonist. The

15 new cytokine should play a role in regulation or development of hematopoietic cells, e.g., lymphoid cells, which affect immunological responses, e.g., inflammation and/or autoimmune disorders. Alternatively, it may affect vascular physiology or development, or neuronal effects.

20 In particular, the cytokine should mediate, in various contexts, cytokine synthesis by the cells, proliferation, etc. Antagonists of IL-B50, such as mutein variants of a naturally occurring form of IL-B50 or blocking antibodies, may provide a selective and powerful way to block immune

25 responses, e.g., in situations as inflammatory or autoimmune responses. See also Samter, et al. (eds.) Immunological Diseases vols. 1 and 2, Little, Brown and Co.

Various abnormal conditions are known in different cell types which will produce IL-B50, e.g., as evaluated by

30 mRNA expression by Northern blot analysis. See Berkow (ed.) The Merck Manual of Diagnosis and Therapy, Merck & Co., Rahway, N.J.; Thorn, et al. Harrison's Principles of Internal Medicine, McGraw-Hill, N.Y.; and Weatherall, et al. (eds.) Oxford Textbook of Medicine, Oxford University Press,

35 Oxford. Many other medical conditions and diseases involve activation by macrophages or monocytes, and many of these

will be responsive to treatment by an agonist or antagonist provided herein. See, e.g., Stites and Terr (eds.; 1991) Basic and Clinical Immunology Appleton and Lange, Norwalk, Connecticut; and Samter, et al. (eds.) Immunological

- 5 Diseases Little, Brown and Co. These problems should be susceptible to prevention or treatment using compositions provided herein.

- IL-B50, antagonists, antibodies, etc., can be purified and then administered to a patient, veterinary or human.
- 10 These reagents can be combined for therapeutic use with additional active or inert ingredients, e.g., in conventional pharmaceutically acceptable carriers or diluents, e.g., immunogenic adjuvants, along with physiologically innocuous stabilizers, excipients, or
- 15 preservatives. These combinations can be sterile filtered and placed into dosage forms as by lyophilization in dosage vials or storage in stabilized aqueous preparations. This invention also contemplates use of antibodies or binding fragments thereof, including forms which are not complement
- 20 binding.

- Drug screening using IL-B50 or fragments thereof can be performed to identify compounds having binding affinity to or other relevant biological effects on IL-B50 functions, including isolation of associated components. Subsequent
- 25 biological assays can then be utilized to determine if the compound has intrinsic stimulating activity and is therefore a blocker or antagonist in that it blocks the activity of the cytokine. Likewise, a compound having intrinsic stimulating activity can activate the signal pathway and is
- 30 thus an agonist in that it simulates the activity of IL-B50. This invention further contemplates the therapeutic use of blocking antibodies to IL-B50 as antagonists and of stimulatory antibodies as agonists. This approach should be particularly useful with other IL-B50 species variants.

- 35 The quantities of reagents necessary for effective therapy will depend upon many different factors, including

1900 1901 1902 1903 1904 1905 1906 1907 1908 1909 1910 1911 1912 1913 1914 1915 1916 1917 1918 1919 1920 1921 1922 1923 1924 1925 1926 1927 1928 1929 1930 1931 1932 1933 1934 1935 1936 1937 1938 1939 1940 1941 1942 1943 1944 1945 1946 1947 1948 1949 1950 1951 1952 1953 1954 1955 1956 1957 1958 1959 1960 1961 1962 1963 1964 1965 1966 1967 1968 1969 1970 1971 1972 1973 1974 1975 1976 1977 1978 1979 1980 1981 1982 1983 1984 1985 1986 1987 1988 1989 1990 1991 1992 1993 1994 1995 1996 1997 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017 2018 2019 2020 2021 2022 2023 2024 2025 2026 2027 2028 2029 2030 2031 2032 2033 2034 2035 2036 2037 2038 2039 2040 2041 2042 2043 2044 2045 2046 2047 2048 2049 2050 2051 2052 2053 2054 2055 2056 2057 2058 2059 2060 2061 2062 2063 2064 2065 2066 2067 2068 2069 2070 2071 2072 2073 2074 2075 2076 2077 2078 2079 2080 2081 2082 2083 2084 2085 2086 2087 2088 2089 2090 2091 2092 2093 2094 2095 2096 2097 2098 2099 2100 2101 2102 2103 2104 2105 2106 2107 2108 2109 2110 2111 2112 2113 2114 2115 2116 2117 2118 2119 2120 2121 2122 2123 2124 2125 2126 2127 2128 2129 2130 2131 2132 2133 2134 2135 2136 2137 2138 2139 2140 2141 2142 2143 2144 2145 2146 2147 2148 2149 2150 2151 2152 2153 2154 2155 2156 2157 2158 2159 2160 2161 2162 2163 2164 2165 2166 2167 2168 2169 2170 2171 2172 2173 2174 2175 2176 2177 2178 2179 2180 2181 2182 2183 2184 2185 2186 2187 2188 2189 2190 2191 2192 2193 2194 2195 2196 2197 2198 2199 2200 2201 2202 2203 2204 2205 2206 2207 2208 2209 2210 2211 2212 2213 2214 2215 2216 2217 2218 2219 2220 2221 2222 2223 2224 2225 2226 2227 2228 2229 2230 2231 2232 2233 2234 2235 2236 2237 2238 2239 2240 2241 2242 2243 2244 2245 2246 2247 2248 2249 2250 2251 2252 2253 2254 2255 2256 2257 2258 2259 2260 2261 2262 2263 2264 2265 2266 2267 2268 2269 2270 2271 2272 2273 2274 2275 2276 2277 2278 2279 2280 2281 2282 2283 2284 2285 2286 2287 2288 2289 2290 2291 2292 2293 2294 2295 2296 2297 2298 2299 2300 2301 2302 2303 2304 2305 2306 2307 2308 2309 2310 2311 2312 2313 2314 2315 2316 2317 2318 2319 2320 2321 2322 2323 2324 2325 2326 2327 2328 2329 2330 2331 2332 2333 2334 2335 2336 2337 2338 2339 2340 2341 2342 2343 2344 2345 2346 2347 2348 2349 2350 2351 2352 2353 2354 2355 2356 2357 2358 2359 2360 2361 2362 2363 2364 2365 2366 2367 2368 2369 2370 2371 2372 2373 2374 2375 2376 2377 2378 2379 2380 2381 2382 2383 2384 2385 2386 2387 2388 2389 2390 2391 2392 2393 2394 2395 2396 2397 2398 2399 2400 2401 2402 2403 2404 2405 2406 2407 2408 2409 2410 2411 2412 2413 2414 2415 2416 2417 2418 2419 2420 2421 2422 2423 2424 2425 2426 2427 2428 2429 2430 2431 2432 2433 2434 2435 2436 2437 2438 2439 2440 2441 2442 2443 2444 2445 2446 2447 2448 2449 2450 2451 2452 2453 2454 2455 2456 2457 2458 2459 2460 2461 2462 2463 2464 2465 2466 2467 2468 2469 2470 2471 2472 2473 2474 2475 2476 2477 2478 2479 2480 2481 2482 2483 2484 2485 2486 2487 2488 2489 2490 2491 2492 2493 2494 2495 2496 2497 2498 2499 2500 2501 2502 2503 2504 2505 2506 2507 2508 2509 2510 2511 2512 2513 2514 2515 2516 2517 2518 2519 2520 2521 2522 2523 2524 2525 2526 2527 2528 2529 2530 2531 2532 2533 2534 2535 2536 2537 2538 2539 2540 2541 2542 2543 2544 2545 2546 2547 2548 2549 2550 2551 2552 2553 2554 2555 2556 2557 2558 2559 2560 2561 2562 2563 2564 2565 2566 2567 2568 2569 2570 2571 2572 2573 2574 2575 2576 2577 2578 2579 2580 2581 2582 2583 2584 2585 2586 2587 2588 2589 2590 2591 2592 2593 2594 2595 2596 2597 2598 2599 2600 2601 2602 2603 2604 2605 2606 2607 2608 2609 2610 2611 2612 2613 2614 2615 2616 2617 2618 2619 2620 2621 2622 2623 2624 2625 2626 2627 2628 2629 2630 2631 2632 2633 2634 2635 2636 2637 2638 2639 2640 2641 2642 2643 2644 2645 2646 2647 2648 2649 2650 2651 2652 2653 2654 2655 2656 2657 2658 2659 2660 2661 2662 2663 2664 2665 2666 2667 2668 2669 2670 2671 2672 2673 2674 2675 2676 2677 2678 2679 2680 2681 2682 2683 2684 2685 2686 2687 2688 2689 2690 2691 2692 2693 2694 2695 2696 2697 2698 2699 2700 2701 2702 2703 2704 2705 2706 2707 2708 2709 2710 2711 2712 2713 2714 2715 2716 2717 2

IL-B50, fragments thereof, and antibodies to it or its fragments, antagonists, and agonists, may be administered
30 directly to the host to be treated or, depending on the size of the compounds, it may be desirable to conjugate them to carrier proteins such as ovalbumin or serum albumin prior to their administration. Therapeutic formulations may be administered in many conventional dosage formulations.
35 While it is possible for the active ingredient to be administered alone, it is preferable to present it as a

pharmaceutical formulation. Formulations typically comprise at least one active ingredient, as defined above, together with one or more acceptable carriers thereof. Each carrier should be both pharmaceutically and physiologically acceptable in the sense of being compatible with the other ingredients and not injurious to the patient. Formulations include those suitable for oral, rectal, nasal, topical, or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. See, e.g., Gilman, et al. (eds. 1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press; and Remington's Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn.; Avis, et al. (eds. 1993) Pharmaceutical Dosage Forms: Parenteral Medications, Dekker, New York; Lieberman, et al. (eds. 1990) Pharmaceutical Dosage Forms: Tablets, Dekker, New York; and Lieberman, et al. (eds. 1990) Pharmaceutical Dosage Forms: Disperse Systems, Dekker, New York. The therapy of this invention may be combined with or used in association with other agents, e.g., other cytokines, including IL-7, or its antagonists.

Both naturally occurring and recombinant forms of the IL-B50s of this invention are particularly useful in kits and assay methods which are capable of screening compounds for binding activity to the proteins. Several methods of automating assays have been developed in recent years so as to permit screening of tens of thousands of compounds in a short period. See, e.g., Fodor, et al. (1991) Science 251:767-773, which describes means for testing of binding affinity by a plurality of defined polymers synthesized on a solid substrate. The development of suitable assays can be greatly facilitated by the availability of large amounts of purified, soluble IL-B50 as provided by this invention.

Other methods can be used to determine the critical residues in IL-B50-IL-B50 receptor interactions. Mutational analysis can be performed, e.g., see Somoza, et al. (1993) J. Exptl. Med. 178:549-558, to determine specific residues critical in the interaction and/or signaling. PHD (Rost and Sander (1994) Proteins 19:55-72) and DSC (King and Sternberg (1996) Protein Sci. 5:2298-2310) can provide secondary structure predictions of α -helix (H), β -strand (E), or coil (L). Helices A and D are most important in receptor interaction, with the D helix the more important region. Helix A would run (SEQ ID NO: 4) in the human from about lys10 to tyr26, helix B would run from about his46 to asn57; helix C would run from about glu68 to trp81; and helix D would run from about asn107 to position about leu127. Surface exposed residues would affect receptor binding, while embedded residues would affect general structure.

For example, antagonists can normally be found once the antigen has been structurally defined, e.g., by tertiary structure data. Testing of potential interacting analogs is now possible upon the development of highly automated assay methods using a purified IL-B50. In particular, new agonists and antagonists will be discovered by using screening techniques described herein. Of particular importance are compounds found to have a combined binding affinity for a spectrum of IL-B50 molecules, e.g., compounds which can serve as antagonists for species variants of IL-B50.

One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant DNA molecules expressing an IL-B50. Cells may be isolated which express an IL-B50 in isolation from other molecules. Such cells, either in viable or fixed form, can be used for standard binding partner binding assays. See also, Parce, et al. (1989) Science 246:243-247; and Owicki, et al. (1990) Proc. Nat'l Acad. Sci. USA 87:4007-4011, which describe sensitive methods to detect cellular responses.

Another technique for drug screening involves an approach which provides high throughput screening for compounds having suitable binding affinity to an IL-B50 and is described in detail in Geysen, European Patent
5 Application 84/03564, published on September 13, 1984. First, large numbers of different small peptide test compounds are synthesized on a solid substrate, e.g., plastic pins or some other appropriate surface, see Fodor, et al. (1991). Then all the pins are reacted with
10 solubilized, unpurified or solubilized, purified IL-B50, and washed. The next step involves detecting bound IL-B50.

Rational drug design may also be based upon structural studies of the molecular shapes of the IL-B50 and other effectors or analogs. Effectors may be other proteins which
15 mediate other functions in response to binding, or other proteins which normally interact with IL-B50, e.g., a receptor. One means for determining which sites interact with specific other proteins is a physical structure determination, e.g., x-ray crystallography or 2 dimensional
20 NMR techniques. These will provide guidance as to which amino acid residues form molecular contact regions, as modeled, e.g., against other cytokine-receptor models. For a detailed description of protein structural determination, see, e.g., Blundell and Johnson (1976) Protein
25 Crystallography, Academic Press, New York.

IX. Kits

This invention also contemplates use of IL-B50 proteins, fragments thereof, peptides, and their fusion
30 products in a variety of diagnostic kits and methods for detecting the presence of another IL-B50 or binding partner. Typically the kit will have a compartment containing either a defined IL-B50 peptide or gene segment or a reagent which recognizes one or the other, e.g., IL-B50 fragments or
35 antibodies.

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A kit for determining the binding affinity of a test compound to an IL-B50 would typically comprise a test compound; a labeled compound, for example a binding partner or antibody having known binding affinity for IL-B50; a
5 source of IL-B50 (naturally occurring or recombinant); and a means for separating bound from free labeled compound, such as a solid phase for immobilizing the molecule. Once compounds are screened, those having suitable binding affinity to the antigen can be evaluated in suitable
10 biological assays, as are well known in the art, to determine whether they act as agonists or antagonists to the IL-B50 signaling pathway. The availability of recombinant IL-B50 polypeptides also provide well defined standards for calibrating such assays.

15 A preferred kit for determining the concentration of, e.g., an IL-B50 in a sample would typically comprise a labeled compound, e.g., binding partner or antibody, having known binding affinity for the antigen, a source of cytokine (naturally occurring or recombinant) and a means for
20 separating the bound from free labeled compound, e.g., a solid phase for immobilizing the IL-B50. Compartments containing reagents, and instructions, will normally be provided.

Antibodies, including antigen binding fragments,
25 specific for the IL-B50 or fragments are useful in diagnostic applications to detect the presence of elevated levels of IL-B50 and/or its fragments. Such diagnostic assays can employ lysates, live cells, fixed cells, immunofluorescence, cell cultures, body fluids, and further
30 can involve the detection of antigens related to the antigen in serum, or the like. Diagnostic assays may be homogeneous (without a separation step between free reagent and antigen-binding partner complex) or heterogeneous (with a separation step). Various commercial assays exist, such as
35 radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), enzyme immunoassay (EIA), enzyme-multiplied

immunoassay technique (EMIT), substrate-labeled fluorescent immunoassay (SLFIA), and the like. See, e.g., Van Vunakis, et al. (1980) Meth Enzymol. 70:1-525; Harlow and Lane (1980) Antibodies: A Laboratory Manual, CSH Press, NY; and
5 Coligan, et al. (eds. 1993) Current Protocols in Immunology, Greene and Wiley, NY.

Anti-idiotypic antibodies may have similar use to diagnose presence of antibodies against an IL-B50, as such may be diagnostic of various abnormal states. For example,
10 overproduction of IL-B50 may result in production of various immunological reactions which may be diagnostic of abnormal physiological states, particularly in proliferative cell conditions such as cancer or abnormal activation or differentiation. Moreover, the distribution pattern
15 available provides information that the cytokine is expressed in pancreatic islets, suggesting the possibility that the cytokine may be involved in function of that organ, e.g., in a diabetes relevant medical condition.

Frequently, the reagents for diagnostic assays are
20 supplied in kits, so as to optimize the sensitivity of the assay. For the subject invention, depending upon the nature of the assay, the protocol, and the label, either labeled or unlabeled antibody or binding partner, or labeled IL-B50 is provided. This is usually in conjunction with other
25 additives, such as buffers, stabilizers, materials necessary for signal production such as substrates for enzymes, and the like. Preferably, the kit will also contain instructions for proper use and disposal of the contents after use. Typically the kit has compartments for each
30 useful reagent. Desirably, the reagents are provided as a dry lyophilized powder, where the reagents may be reconstituted in an aqueous medium providing appropriate concentrations of reagents for performing the assay.

Many of the aforementioned constituents of the drug
35 screening and the diagnostic assays may be used without modification or may be modified in a variety of ways. For

example, labeling may be achieved by covalently or non-covalently joining a moiety which directly or indirectly provides a detectable signal. In any of these assays, the binding partner, test compound, IL-B50, or antibodies thereto can be labeled either directly or indirectly. Possibilities for direct labeling include label groups: radiolabels such as ^{125}I , enzymes (U.S. Pat. No. 3,645,090) such as peroxidase and alkaline phosphatase, and fluorescent labels (U.S. Pat. No. 3,940,475) capable of monitoring the change in fluorescence intensity, wavelength shift, or fluorescence polarization. Possibilities for indirect labeling include biotinylation of one constituent followed by binding to avidin coupled to one of the above label groups.

There are also numerous methods of separating the bound from the free IL-B50, or alternatively the bound from the free test compound. The IL-B50 can be immobilized on various matrixes followed by washing. Suitable matrixes include plastic such as an ELISA plate, filters, and beads. See, e.g., Coligan, et al. (eds. 1993) Current Protocols in Immunology, Vol. 1, Chapter 2, Greene and Wiley, NY. Other suitable separation techniques include, without limitation, the fluorescein antibody magnetizable particle method described in Rattle, et al. (1984) Clin. Chem. 30:1457-1461, and the double antibody magnetic particle separation as described in U.S. Pat. No. 4,659,678.

Methods for linking proteins or their fragments to the various labels have been extensively reported in the literature and do not require detailed discussion here. Many of the techniques involve the use of activated carboxyl groups either through the use of carbodiimide or active esters to form peptide bonds, the formation of thioethers by reaction of a mercapto group with an activated halogen such as chloroacetyl, or an activated olefin such as maleimide, for linkage, or the like. Fusion proteins will also find use in these applications.

Another diagnostic aspect of this invention involves use of oligonucleotide or polynucleotide sequences taken from the sequence of an IL-B50. These sequences can be used as probes for detecting levels of the IL-B50 message in samples from patients suspected of having an abnormal condition, e.g., inflammatory or autoimmune. Since the cytokine may be a marker or mediator for activation, it may be useful to determine the numbers of activated cells to determine, e.g., when additional therapy may be called for, e.g., in a preventative fashion before the effects become and progress to significance. The preparation of both RNA and DNA nucleotide sequences, the labeling of the sequences, and the preferred size of the sequences has received ample description and discussion in the literature. See, e.g., Langer-Safer, et al. (1982) Proc. Nat'l. Acad. Sci. 79:4381-4385; Caskey (1987) Science 236:962-967; and Wilchek et al. (1988) Anal. Biochem. 171:1-32.

Diagnostic kits which also test for the qualitative or quantitative expression of other molecules are also contemplated. Diagnosis or prognosis may depend on the combination of multiple indications used as markers. Thus, kits may test for combinations of markers. See, e.g., Viallet, et al. (1989) Progress in Growth Factor Res. 1:89-97. Other kits may be used to evaluate other cell subsets.

X. Isolating an IL-B50 Receptor

Having isolated a ligand of a specific ligand-receptor interaction, methods exist for isolating the receptor. See, Gearing, et al. (1989) EMBO J. 8:3667-3676. For example, means to label the IL-B50 cytokine without interfering with the binding to its receptor can be determined. For example, an affinity label can be fused to either the amino- or carboxyl-terminus of the ligand. Such label may be a FLAG epitope tag, or, e.g., an Ig or Fc domain. An expression library can be screened for specific binding of the cytokine, e.g., by cell sorting, or other screening to

detect subpopulations which express such a binding component. See, e.g., Ho, et al. (1993) Proc. Nat'l Acad. Sci. USA 90:11267-11271; and Liu, et al. (1994) J. Immunol. 152:1821-29. Alternatively, a panning method may be used.

- 5 See, e.g., Seed and Aruffo (1987) Proc. Nat'l Acad. Sci. USA 84:3365-3369.

Protein cross-linking techniques with label can be applied to isolate binding partners of the IL-B50 cytokine. This would allow identification of proteins which
10 specifically interact with the cytokine, e.g., in a ligand-receptor like manner. It is predicted that the IL-B50 will bind to the IL-7R alpha subunit, but this is likely to the beta subunit in the IL-B50 receptor. Thus, another subunit would serve as the alpha receptor subunit for the IL-B50.

- 15 Early experiments will be performed, as predicted, to determine whether the known IL-7 receptor components are involved in response(s) to IL-B50. It is also quite possible that these functional receptor complexes may share many or all components with an IL-B50 receptor complex,
20 either a specific receptor subunit or an accessory receptor subunit.

- Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific
25 embodiments described herein are offered by way of example only, and the invention is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled.

EXAMPLES

I. General Methods

- Many of the standard methods below are described or referenced, e.g., in Maniatis, et al. (1982) Molecular Cloning, A Laboratory Manual Cold Spring Harbor Laboratory, Cold Spring Harbor Press, NY; Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual (2d ed.) Vols. 1-3, CSH Press, NY; Ausubel, et al., Biology Greene Publishing Associates, Brooklyn, NY; or Ausubel, et al. (1987 and Supplements) Current Protocols in Molecular Biology Wiley/Greene, NY; Innis, et al. (eds. 1990) PCR Protocols: A Guide to Methods and Applications Academic Press, NY.
- Methods for protein purification include such methods as ammonium sulfate precipitation, column chromatography, electrophoresis, centrifugation, crystallization, and others. See, e.g., Ausubel, et al. (1987 and periodic supplements); Deutscher (1990) "Guide to Protein Purification," Methods in Enzymology vol. 182, and other volumes in this series; Coligan, et al. (1995 and supplements) Current Protocols in Protein Science John Wiley and Sons, New York, NY; Matsudaira (ed. 1993) A Practical Guide to Protein and Peptide Purification for Microsequencing, Academic Press, San Diego, CA; and manufacturer's literature on use of protein purification products, e.g., Pharmacia, Piscataway, NJ, or Bio-Rad, Richmond, CA. Combination with recombinant techniques allow fusion to appropriate segments (epitope tags), e.g., to a FLAG sequence or an equivalent which can be fused, e.g., via a protease-removable sequence. See, e.g., Hochuli (1989) Chemische Industrie 12:69-70; Hochuli (1990) "Purification of Recombinant Proteins with Metal Chelate Absorbent" in Setlow (ed.) Genetic Engineering, Principle and Methods 12:87-98, Plenum Press, NY; and Crowe, et al. (1992) QIAexpress: The High Level Expression & Protein Purification System QUIAGEN, Inc., Chatsworth, CA.

Standard immunological techniques are described, e.g., in Hertenberg, et al. (eds. 1996) Weir's Handbook of Experimental Immunology vols. 1-4, Blackwell Science; Coligan (1991) Current Protocols in Immunology Wiley/Greene, NY; and Methods in Enzymology vols. 70, 73, 74, 84, 92, 93, 108, 116, 121, 132, 150, 162, and 163. Cytokine assays are described, e.g., in Thomson (ed. 1998) The Cytokine Handbook (3d ed.) Academic Press, San Diego; Mire-Sluis and Thorpe (1998) Cytokines Academic Press, San Diego; Metcalf and Nicola (1995) The Hematopoietic Colony Stimulating Factors Cambridge University Press; and Aggarwal and Gutterman (1991) Human Cytokines Blackwell Pub.

Assays for vascular biological activities are well known in the art. They will cover angiogenic and angiostatic activities in tumor, or other tissues, e.g., arterial smooth muscle proliferation (see, e.g., Koyoma, et al. (1996) Cell 87:1069-1078), monocyte adhesion to vascular epithelium (see McEvoy, et al. (1997) J. Exp. Med. 185:2069-2077), etc. See also Ross (1993) Nature 362:801-809; Rekhter and Gordon (1995) Am. J. Pathol. 147:668-677; Thyberg, et al. (1990) Atherosclerosis 10:966-990; and Gumbiner (1996) Cell 84:345-357.

Assays for neural cell biological activities are described, e.g., in Wouterlood (ed. 1995) Neuroscience Protocols modules 10, Elsevier; Methods in Neurosciences Academic Press; and Neuromethods Humana Press, Totowa, NJ. Methodology of developmental systems is described, e.g., in Meisami (ed.) Handbook of Human Growth and Developmental Biology CRC Press; and Chrispeels (ed.) Molecular Techniques and Approaches in Developmental Biology Interscience.

FACS analyses are described in Melamed, et al. (1990) Flow Cytometry and Sorting Wiley-Liss, Inc., New York, NY; Shapiro (1988) Practical Flow Cytometry Liss, New York, NY; and Robinson, et al. (1993) Handbook of Flow Cytometry Methods Wiley-Liss, New York, NY.

II. Cloning of Human IL-B50

The sequence of the gene is provided in Table 1. The sequence is derived from a cDNA library made from testes. The sequence is quite rare, not being found with frequency
5 in available sequence databases. This sequence allows preparation of PCR primers, or probes, to determine cellular distribution of the gene. The sequence allows isolation of genomic DNA which encode the message.

Using the probe or PCR primers, various tissues or
10 cell types are probed to determine cellular distribution. PCR products are cloned using, e.g., a TA cloning kit (Invitrogen). The resulting cDNA plasmids are sequenced from both termini on an automated sequencer (Applied Biosystems).

15 III. Cellular Expression of IL-B50

An appropriate probe or primers specific for cDNA encoding primate IL-B50 are prepared. Typically, the probe is labeled, e.g., by random priming.

20 Southern Analysis: DNA (5 µg) from a primary amplified cDNA library was digested with appropriate restriction enzymes to release the inserts, run on a 1% agarose gel and transferred to a nylon membrane (Schleicher and Schuell, Keene, NH).

25 Samples for human mRNA isolation may include:
peripheral blood mononuclear cells (monocytes, T cells, NK cells, granulocytes, B cells), resting (T100); peripheral blood mononuclear cells, activated with anti-CD3 for 2, 6, 12 h pooled (T101); T cell, TH0 clone Mot 72, resting
30 (T102); T cell, TH0 clone Mot 72, activated with anti-CD28 and anti-CD3 for 3, 6, 12 h pooled (T103); T cell, TH0 clone Mot 72, anergic treated with specific peptide for 2, 7, 12 h pooled (T104); T cell, TH1 clone HY06, resting (T107); T
cell, TH1 clone HY06, activated with anti-CD28 and anti-CD3
35 for 3, 6, 12 h pooled (T108); T cell, TH1 clone HY06, anergic treated with specific peptide for 2, 6, 12 h pooled

(T109); T cell, TH2 clone HY935, resting (T110); T cell, TH2 clone HY935, activated with anti-CD28 and anti-CD3 for 2, 7, 12 h pooled (T111); T cell tumor lines Jurkat and Hut78, resting (T117); T cell clones, pooled AD130.2, Tc783.12, Tc783.13, Tc783.58, Tc782.69, resting (T118); T cell random $\gamma\delta$ T cell clones, resting (T119); CD28- T cell clone; Splenocytes, resting (B100); Splenocytes, activated with anti-CD40 and IL-4 (B101); B cell EBV lines pooled WT49, RSB, JY, CVIR, 721.221, RM3, HSY, resting (B102); B cell line JY, activated with PMA and ionomycin for 1, 6 h pooled (B103); NK 20 clones pooled, resting (K100); NK 20 clones pooled, activated with PMA and ionomycin for 6 h (K101); NKL clone, derived from peripheral blood of LGL leukemia patient, IL-2 treated (K106); hematopoietic precursor line TF1, activated with PMA and ionomycin for 1, 6 h pooled (C100); U937 premonocytic line, resting (M100); U937 premonocytic line, activated with PMA and ionomycin for 1, 6 h pooled (M101); elutriated monocytes, activated with LPS, IFN γ , anti-IL-10 for 1, 2, 6, 12, 24 h pooled (M102); elutriated monocytes, activated with LPS, IFN γ , IL-10 for 1, 2, 6, 12, 24 h pooled (M103); elutriated monocytes, activated with LPS, IFN γ , anti-IL-10 for 4, 16 h pooled (M106); elutriated monocytes, activated with LPS, IFN γ , IL-10 for 4, 16 h pooled (M107); elutriated monocytes, activated LPS for 1 h (M108); elutriated monocytes, activated LPS for 6 h (M109); DC 70% CD1a+, from CD34+ GM-CSF, TNF α 12 days, resting (D101); DC 70% CD1a+, from CD34+ GM-CSF, TNF α 12 days, activated with PMA and ionomycin for 1 hr (D102); DC 70% CD1a+, from CD34+ GM-CSF, TNF α 12 days, activated with PMA and ionomycin for 6 hr (D103); DC 95% CD1a+, from CD34+ GM-CSF, TNF α 12 days FACS sorted, activated with PMA and ionomycin for 1, 6 h pooled (D104); DC 95% CD14+, ex CD34+ GM-CSF, TNF α 12 days FACS sorted, activated with PMA and ionomycin 1, 6 hr pooled (D105); DC CD1a+ CD86+, from CD34+ GM-CSF, TNF α 12 days FACS sorted, activated with PMA and ionomycin for 1, 6 h pooled (D106);

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DC from monocytes GM-CSF, IL-4 5 days, resting (D107); DC from monocytes GM-CSF, IL-4 5 days, resting (D108); DC from monocytes GM-CSF, IL-4 5 days, activated LPS 4, 16 h pooled (D109); DC from monocytes GM-CSF, IL-4 5 days, activated TNF α , monocyte supe for 4, 16 h pooled (D110); epithelial cells, unstimulated; epithelial cells, IL-1 β activated; lung fibroblast sarcoma line MRC5, activated with PMA and ionomycin for 1, 6 h pooled (C101); kidney epithelial carcinoma cell line CHA, activated with PMA and ionomycin for 1, 6 h pooled (C102).

A mouse counterpart will be identified, and its distributions will be similarly evaluated. Samples for mouse mRNA isolation can include: resting mouse fibroblastic L cell line (C200); Braf:ER (Braf fusion to estrogen receptor) transfected cells, control (C201); Mel14+ naive T cells from spleen, resting (T209); Mel14+ naive T cells from spleen, stimulated with IFN γ , IL-12, and anti IL-4 to polarize to TH1 cells, exposed to IFN γ and IL-4 for 6, 12, 24 h, pooled (T210); Mel14+ naive T cells from spleen, stimulated with IL-4 and anti IFN γ to polarize to Th2 cells, exposed to IL-4 and anti IFN γ for 6, 13, 24 h, pooled (T211); T cells, TH1 polarized (Mel14 bright, CD4+ cells from spleen, polarized for 7 days with IFN- γ and anti IL-4; T200); T cells, TH2 polarized (Mel14 bright, CD4+ cells from spleen, polarized for 7 days with IL-4 and anti-IFN- γ ; T201); T cells, highly TH1 polarized 3x from transgenic Balb/C (see Openshaw, et al. (1995) J. Exp. Med. 182:1357-1367; activated with anti-CD3 for 2, 6, 24 h pooled; T202); T cells, highly TH2 polarized 3x from transgenic Balb/C (activated with anti-CD3 for 2, 6, 24 h pooled (T203); T cells, highly TH1 polarized 3x from transgenic C57 bl/6 (activated with anti-CD3 for 2, 6, 24 h pooled; T212); T cells, highly TH2 polarized 3x from transgenic C57 bl/6 (activated with anti-CD3 for 2, 6, 24 h pooled; T213); T cells, highly TH1 polarized (naive CD4+ T cells from transgenic Balb/C, polarized 3x with IFN γ , IL-12, and anti-

IL-4; stimulated with IGIF, IL-12, and anti IL-4 for 6, 12, 24 h, pooled); CD44- CD25+ pre T cells, sorted from thymus (T204); TH1 T cell clone D1.1, resting for 3 weeks after last stimulation with antigen (T205); TH1 T cell clone D1.1, 10 µg/ml ConA stimulated 15 h (T206); TH2 T cell clone CDC35, resting for 3 weeks after last stimulation with antigen (T207); TH2 T cell clone CDC35, 10 µg/ml ConA stimulated 15 h (T208); unstimulated B cell line CH12 (B201); unstimulated mature B cell leukemia cell line A20 (B200); unstimulated large B cells from spleen (B202); B cells from total spleen, LPS activated (B203); metrizamide enriched dendritic cells from spleen, resting (D200); dendritic cells from bone marrow, resting (D201); unstimulated bone marrow derived dendritic cells depleted with anti B220, anti CD3, and anti Class II, cultured in GM-CSF and IL-4 (D202); bone marrow derived dendritic cells depleted with anti B220, anti CD3, and anti Class II, cultured in GM-CSF and IL-4, stimulated with anti CD40 for 1, 5 d, pooled (D203); monocyte cell line RAW 264.7 activated with LPS 4 h (M200); bone-marrow macrophages derived with GM and M-CSF (M201); bone-marrow macrophages derived with GM-CSF, stimulated with LPS, IFN γ , and IL-10 for 24 h (M205); bone-marrow macrophages derived with GM-CSF, stimulated with LPS, IFN γ , and anti IL-10 for 24 h (M206); peritoneal macrophages (M207); macrophage cell line J774, resting (M202); macrophage cell line J774 + LPS + anti-IL-10 at 0.5, 1, 3, 6, 12 h pooled (M203); macrophage cell line J774 + LPS + IL-10 at 0.5, 1, 3, 5, 12 h pooled (M204); unstimulated mast cell lines MC-9 and MCP-12 (M208); immortalized endothelial cell line derived from brain microvascular endothelial cells, unstimulated (E200); immortalized endothelial cell line derived from brain microvascular endothelial cells, stimulated overnight with TNF α (E201); immortalized endothelial cell line derived from brain microvascular endothelial cells, stimulated overnight with TNF α (E202); immortalized endothelial cell

line derived from brain microvascular endothelial cells, stimulated overnight with TNF α and IL-10 (E203); total aorta from wt C57 bl/6 mouse; total aorta from 5 month ApoE KO mouse (X207); total aorta from 12 month ApoE KO mouse (X207); wt thymus (O214); total thymus, rag-1 (O208); total kidney, rag-1 (O209); total kidney, NZ B/W mouse; and total heart, rag-1 (O202). High signal was detected in the monocyte cell line RAW 264.7 activated with LPS 4 h (M200); T cells, highly TH1 polarized 3x from transgenic C57 bl/6 (activated with anti-CD3 for 2, 6, 24 h pooled; T212); and T cells, highly TH1 polarized (naive CD4+ T cells from transgenic Balb/C, polarized 3x with IFN γ , IL-12, and anti-IL-4; stimulated with IGIF, IL-12, and anti IL-4 for 6, 12, 24 h, pooled).

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IV. Chromosome mapping of IL-B50

An isolated cDNA encoding the IL-B50 is used. Chromosome mapping is a standard technique. See, e.g., BIOS Laboratories (New Haven, CT) and methods for using a mouse somatic cell hybrid panel with PCR.

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V. Purification of IL-B50 Protein

Multiple transfected cell lines are screened for one which expresses the cytokine at a high level compared with other cells. Various cell lines are screened and selected for their favorable properties in handling. Natural IL-B50 can be isolated from natural sources, or by expression from a transformed cell using an appropriate expression vector. Higher efficiency of secretion has been achieved by use of a heterologous signal sequence. Purification of the expressed protein is achieved by standard procedures, or may be combined with engineered means for effective purification at high efficiency from cell lysates or supernatants. FLAG or His₆ segments can be used for such purification features. Alternatively, affinity chromatography may be used with specific antibodies, see below.

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Protein is produced in coli, insect cell, or mammalian expression systems, as desired.

VI. Isolation of Homologous IL-B50 Genes

5 The IL-B50 cDNA, or other species counterpart
sequence, can be used as a hybridization probe to screen a
library from a desired source, e.g., a primate cell cDNA
library. Many different species can be screened both for
10 stringency necessary for easy hybridization, and for
presence using a probe. Appropriate hybridization
conditions will be used to select for clones exhibiting
specificity of cross hybridization.

Screening by hybridization using degenerate probes
based upon the peptide sequences will also allow isolation
15 of appropriate clones. Alternatively, use of appropriate
primers for PCR screening will yield enrichment of
appropriate nucleic acid clones.

Similar methods are applicable to isolate either
species, polymorphic, or allelic variants. Species
20 variants are isolated using cross-species hybridization
techniques based upon isolation of a full length isolate or
fragment from one species as a probe.

Alternatively, antibodies raised against human IL-B50
will be used to screen for cells which express cross-
25 reactive proteins from an appropriate, e.g., cDNA library.
The purified protein or defined peptides are useful for
generating antibodies by standard methods, as described
above. Synthetic peptides or purified protein are
presented to an immune system to generate monoclonal or
30 polyclonal antibodies. See, e.g., Coligan (1991) Current
Protocols in Immunology Wiley/Greene; and Harlow and Lane
(1989) Antibodies: A Laboratory Manual Cold Spring Harbor
Press. The resulting antibodies are used for screening,
purification, or diagnosis, as described.

VII. Preparation of antibodies specific for IL-B50

Synthetic peptides or purified protein are presented to an immune system to generate monoclonal or polyclonal antibodies. See, e.g., Coligan (1991) Current Protocols in Immunology Wiley/Greene; and Harlow and Lane (1989) Antibodies: A Laboratory Manual Cold Spring Harbor Press. Polyclonal serum, or hybridomas may be prepared. In appropriate situations, the binding reagent is either labeled as described above, e.g., fluorescence or otherwise, or immobilized to a substrate for panning methods. Immunoselection, absorptions, depletions, and related techniques are available to prepare selective reagents, e.g., exhibiting the desired spectrum of selectivity for binding.

VIII. Evaluation of Breadth of Biological Functions

Biological activities of IL-B50 are tested, based, in part, on the sequence and structural homology between IL-B50 and IL-7. Initially, assays that show biological activities of IL-7 are examined.

A. Effects on proliferation/differentiation of progenitor cells

The effect on proliferation or differentiation of various cell types are evaluated with various concentrations of cytokine. A dose response analysis is performed, in certain cases in combination with the related cytokine IL-7 and/or stem cell factor.

In particular, IL-7 exhibits strong effects on lymphopoietic development and differentiation. The IL-B50 will be tested on cord blood cells to see if it has effects on proliferation or differentiation of early progenitor cells derived therefrom. Preferably, the cells are early precursor cells, e.g., stem cells, originating from, e.g., cord blood, bone marrow, thymus, spleen, or CD34+ progenitor cells. See, e.g., Eddington and Lotze (1998).

B. Effects of IL-B50 on proliferation of human peripheral blood mononuclear cells (PBMC)

Total PBMC are isolated from buffy coats of normal healthy donors by centrifugation through ficoll-hypaque as described (Boyum, et al.). PBMC are cultured in 200 µl Yssel's medium (Gemini Bioproducts, Calabasas, CA) containing 1% human AB serum in 96 well plates (Falcon, Becton-Dickinson, NJ) in the absence or presence of IL-B50. Cells are cultured in medium alone or in combination with 100 U/ml IL-2 (R&D Systems) for 120 hours. 3H-Thymidine (0.1 mCi) is added during the last six hours of culture and 3H-Thymidine incorporation determined by liquid scintillation counting.

The native, recombinant, and fusion proteins would be tested for agonist and antagonist activity in many other biological assay systems, e.g., on T-cells, B-cells, NK, macrophages, dendritic cells, hematopoietic progenitors, etc.

IL-B50 is evaluated for agonist or antagonist activity on transfected cells expressing IL-7 receptor and controls.

IL-B50 is evaluated for effect in macrophage/dendritic cell activation and antigen presentation assays, T cell cytokine production and proliferation in response to antigen or allogeneic stimulus. See, e.g., de Waal Malefyt et al. (1991) J. Exp. Med. 174:1209-1220; de Waal Malefyt et al. (1991) J. Exp. Med. 174:915-924; Fiorentino, et al. (1991) J. Immunol. 147, 3815-3822; Fiorentino, et al. (1991) J. Immunol. 146:3444-3451; and Groux, et al. (1996) J. Exp. Med. 184:19-29.

IL-B50 will also be evaluated for effects on NK cell stimulation. Assays may be based, e.g., on Hsu, et al. (1992) Internat. Immunol. 4:563-569; and Schwarz, et al. (1994) J. Immunother. 16:95-104. Other assays are applied to evaluate effects on cytotoxic T cells and LAK cells. See, e.g., Namien and Mire-Sluis (1998).

5 including IgG2 and IgA2 switch factor assays. Note that, unlike COS7 supernatants, NIH3T3 and COP supernatants apparently do not interfere with human B cell assays.

Monocytes are purified by negative selection from peripheral blood mononuclear cells of normal healthy donors. Briefly, 3×10^8 ficoll banded mononuclear cells are incubated on ice with a cocktail of monoclonal antibodies (Becton-Dickinson; Mountain View, CA) consisting, e.g., of 200 μ l of α CD2 (Leu-5A), 200 μ l of α CD3 (Leu-4), 100 μ l of α CD8 (Leu 2a), 100 μ l of α CD19 (Leu-12), 100 μ l of α CD20 (Leu-16), 100 μ l of α CD56 (Leu-19), 100 μ l of α CD67 (IOM 67; Immunotech, Westbrook, ME), and anti-glycophorin antibody (10F7MN, ATCC, Rockville, MD). Antibody bound cells are washed and then incubated with sheep anti-mouse IgG coupled magnetic beads (Dynal, Oslo, Norway) at a bead to cell ratio of 20:1. Antibody bound cells are separated from monocytes by application of a magnetic field.

25 Subsequently, human monocytes are cultured in Yssel's medium (Gemini Bioproducts, Calabasas, CA) containing 1% human AB serum in the absence or presence of IL-B50, IL-6, G-CSF or combinations.

Analyses of the expression of cell surface molecules can be performed by direct immunofluorescence. For example, 2×10^5 purified human monocytes are incubated in phosphate buffered saline (PBS) containing 1% human serum on ice for 20 minutes. Cells are pelleted at $200 \times g$. Cells are resuspended in 20 ml PE or FITC labeled mAb. Following an additional 20 minute incubation on ice, cells are washed in PBS containing 1% human serum followed by two washes in PBS alone. Cells are fixed in PBS containing 1%

paraformaldehyde and analyzed on FACScan flow cytometer (Becton Dickinson; Mountain View, CA). Exemplary mAbs are used, e.g.: CD11b (anti-mac1), CD11c (a gp150/95), CD14 (Leu-M3), CD54 (Leu 54), CD80 (anti-BB1/B7), HLA-DR (L243) from Becton-Dickinson and CD86 (FUN 1; Pharmingen), CD64 (32.2; Medarex), CD40 (mAb89; Schering-Plough France).

D. Effects of IL-B50 on cytokine production by human monocytes

Human monocytes are isolated as described and cultured in Yssel's medium (Gemini Bioproducts, Calabasas, CA) containing 1% human AB serum in the absence or presence of IL-B50 (1/100 dilution baculovirus expressed material). In addition, monocytes are stimulated with LPS (E. coli 0127:B8 Difco) in the absence or presence of IL-B50 and the concentration of cytokines (IL-1 β , IL-6, TNF α , GM-CSF, and IL-10) in the cell culture supernatant determined by ELISA.

For intracytoplasmic staining for cytokines, monocytes are cultured (1 million/ml) in Yssel's medium in the absence or presence of IL-B50 and LPS (E. coli 0127:B8 Difco) and 10 mg/ml Brefeldin A (Epicentre technologies Madison WI) for 12 hrs. Cells are washed in PBS and incubated in 2% formaldehyde/PBS solution for 20 minutes at RT. Subsequently cells are washed, resuspended in permeabilization buffer (0.5% saponin (Sigma) in PBS/BSA (0.5%) /Azide (1 mM)) and incubated for 20 minutes at RT. Cells (2×10^5) are centrifuged and resuspended in 20 ml directly conjugated anti-cytokine mAbs diluted 1:10 in permeabilization buffer for 20 minutes at RT. The following antibodies can be used: IL-1 α -PE (364-3B3-14); IL-6-PE (MQ2-13A5); TNF α -PE (MAb11); GM-CSF-PE (BVD2-21C11); and IL-12-PE (C11.5.14; Pharmingen San Diego, CA). Subsequently, cells are washed twice in permeabilization buffer and once in PBS/BSA/Azide and analyzed on FACScan flow cytometer (Becton Dickinson; Mountain View, CA).

Transgenic mice can be generated by standard methods. Such animals are useful to determine the effects of deletion of the gene, in specific tissues, or completely throughout the organism. Such may provide interesting insight into development of the animal or particular tissues in various stages. Moreover, the effect on various responses to biological stress can be evaluated. See, e.g., Hogan, et al. (1995) Manipulating the Mouse Embryo: A Laboratory

10 Manual (2d ed.) Cold Spring Harbor Laboratory Press.

All references cited herein are incorporated herein by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled.

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